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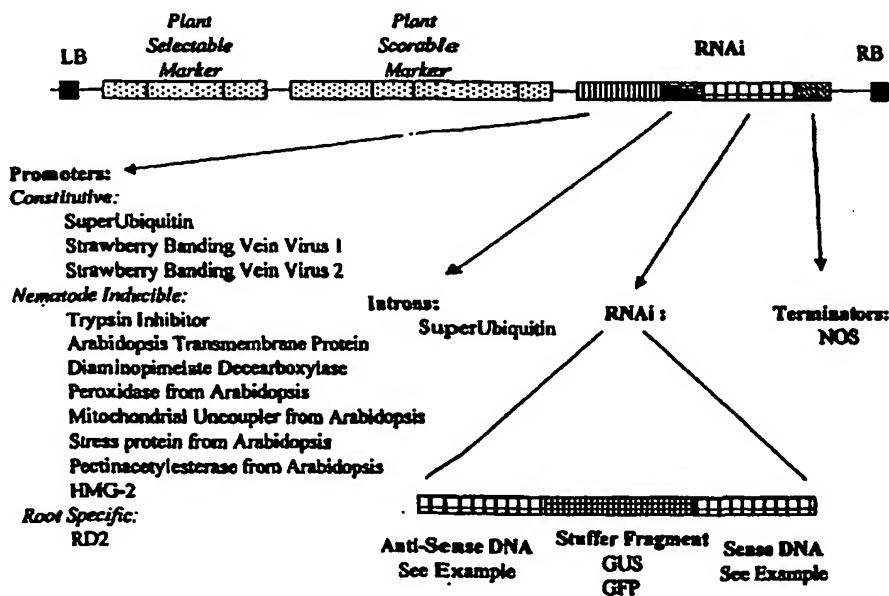
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[Continued on next page]

(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES



(57) Abstract: The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides RNAi molecules, polynucleotide sequences, and methods of using these sequences in nematode control.

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## DESCRIPTION

### MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES

#### Background of the Invention

[0001] Plant parasitic nematodes, such as root-knot nematodes (*Meloidogyne* species) and cyst nematodes (*Globodera* and *Heterodera*), attack nearly every food crop, and are among the world's most damaging agricultural pests. For example, root-knot nematodes parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts.

[0002] Nematodes cause millions of dollars of damage each year to turf grasses, ornamental plants, and food crops. Efforts to eliminate or minimize damage caused by nematodes in agricultural settings have typically involved the use of soil fumigation with materials such as chloropicrin, methyl bromide, and dazomet, which volatilize to spread the active ingredient throughout the soil. Such fumigation materials can be highly toxic and may create an environmental hazard. Various non-fumigant chemicals have also been used, but these too create serious environmental problems and can be highly toxic to humans.

[0003] Some research articles have been published concerning the effects of  $\delta$ -endotoxins from *B. thuringiensis* species on the viability of nematodes. See, for example, Bottjer, Bone and Gill ([1985] *Experimental Parasitology* 60:239-244); Ignoffo and Dropkin (Ignoffo, C.M., Dropkin, V.H. [1977] *J. Kans. Entomol. Soc.* 50:394-398); and Ciordia, H. and W.E. Bizzell ([1961] *Jour. of Parasitology* 47:41 [abstract]). Several patents have issued describing the control of nematodes with *B.t.* See, for example, U.S. Patent Nos. 4,948,734; 5,093,120; 5,281,530; 5,426,049; 5,439,881; 5,236,843; 5,322,932; 5,151,363; 5,270,448; 5,350,577; 5,667,993; and 5,670,365. The development of resistance by insects to *B.t.* toxins is one obstacle to the successful use of such toxins.

[0004] The pesticidal activity of avermectins is well known. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones. They can be divided into four major compounds: A<sub>1a</sub>, A<sub>2a</sub>, B<sub>1a</sub>, and B<sub>2a</sub>; and four minor compounds: A<sub>1b</sub>, A<sub>2b</sub>, B<sub>1b</sub>, and B<sub>2b</sub>. The isolation and purification of these compounds is also described in U.S. Patent No. 4,310,519, issued January 12, 1982. Avermectin B<sub>2a</sub> is active against the root-knot nematode, *Meloidogyne incognita*. It is reported to be 10-30 times as potent as commercial contact nematicides when incorporated into soil at 0.16-0.25 kg/ha (Boyce Thompson Institute for Plant Research 58th Annual Report [1981]; Putter, I. *et al.* [1981] "Avermectins: Novel Insecticides, Acaracides, and Nematicides from a Soil Microorganism," *Experientia* 37:963-964). Avermectin B<sub>2a</sub> is not toxic to tomatoes or cucumbers at rates of up to 10 kg/ha.

[0005] Fatty acids are a class of natural compounds which occur abundantly in nature and which have interesting and valuable biological activities. Tarjan and Cheo (Tarjan, A.C., P.C. Cheo [1956] "Nematocidal Value of Some Fatty Acids," Bulletin 332, Contribution 884, Agricultural Experiment Station, University of Rhode Island, Kingston, 41 pp.) report the activity of certain fatty acids against nematodes. In 1977 Sitaramaiah and Singh (Sitaramaiah, K., R.S. Singh [1977] *Indian J. Nematol.* 7:58-65) also examined the response of nematodes to fatty acids. The results of these tests with short chain acids were equivocal, showing nematode-inhibitory action in some instances and stimulatory activity in other instances. Phytotoxicity of these acids was observed at higher concentrations. The short chain fatty acids were also examined by Malik and Jairajpuri (Malik, Z., M.S. Jairajpuri [1977] *Nematol. medit.* 12:73-79), who observed nematode toxicity at high concentrations of the fatty acids.

[0006] Notwithstanding the foregoing (some of the limitations of and problems associated with these approaches are discussed above), there is a need for safe and effective alternatives for controlling nematodes.

[0007] One method for disrupting normal cellular processes is by the use double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). When RNAi corresponding to a sense and antisense sequence of a target mRNA is introduced into a cell, the targeted mRNA is degraded and protein translation of that message is stopped. Although not yet fully understood, the mechanism of this post-transcriptional gene



silencing appears to be at least partially due to the generation of small RNA molecules, about 21 - 25 nucleotides in length, that correspond to the sense and antisense pieces of the RNAi introduced into the cell (Bass, B. L. [2000] "Double-stranded RNA as a template for gene silencing" *Cell* 101:235-238).

[0008] The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. A recent example of the use of RNAi; to inhibit genetic function in plants used *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* (Chuang, C.-F. and E. M. Meyerowitz [2000] "Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*" *Proc. Natl. Acad. Sci. USA* 97:4985-4990). Chuang *et al.* describe the construction of vectors delivering variable levels of RNAi targeted to each of four genes involved in floral development. Severity of abnormal flower development varied between transgenic lines. For one of the genes, AGAMOUS (AG), a strong correlation existed between declining accumulation of mRNA and increasingly severe phenotypes, suggesting that AG-specific endogenous mRNA is the target of RNAi.

#### Brief Summary of the Invention

[0009] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences that encode nematode genes, RNAi that selectively targets mRNA transcripts of these essential nematode genes, and methods of using these sequences in nematode control strategies. Such sequences for use according to the subject invention are summarized in Appendix 1. RNAi molecules disclosed herein can be used to inhibit the expression of one or more of these genes in nematodes.

### Brief Description of the Drawings

[00010] Figure 1: Modular Binary Construct System (MBCS): A series of six, 8-base cutter restriction enzyme sites has been placed between the left and right T<sub>i</sub> borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid.

[00011] Figure 2: An exemplary shuttle vector created for cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites.

[00012] Figure 3: An exemplary shuttle vector with exemplary inserts.

[00013] Figure 4: A suggested RNAi binary vector with exemplary inserts.

[00014] Figure 5: Exemplary selectable markers for MBCS.

[00015] Figure 6: Exemplary scorable markers for MCBS.

[00016] Figure 7: Exemplary RNAi binary vector.

[00017] Figure 8: Exemplary RNAi shuttle vector.

### Brief Description of the Sequences

[00018] Brief Description of the Sequences can be found in Appendix I.

### Detailed Disclosure of the Invention

[00019] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences and methods of using these sequences in nematode control strategies. A preferred method for controlling nematodes according to the subject invention provides materials and methods for controlling nematodes by using double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). The terms RNAi and RNAi are used interchangeably herein unless otherwise noted.

[00020] In one embodiment of the invention, RNAi molecules are provided which are useful in methods of killing nematodes and/or inhibiting their growth, development, parasitism or reproduction. RNAi molecules of the invention are also useful for the regulation of levels of specific mRNA in nematodes.

[00021] dsRNA (RNAi) typically comprises a polynucleotide sequence identical to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide

sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker (stuffer) sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker (stuffer) sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules.

[00022] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[00023] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[00024] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA

may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[00025] Preferably and most conveniently, RNAi can be targeted to an entire polynucleotide sequence of a gene set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides summarized in Appendix 1. The homology is preferably greater than 90% and is most preferably greater than 95%.

[00026] Fragments of genes can also be targeted. These fragments are typically in the approximate size range of about 20 nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. However, other size ranges can also be used. For example, using a *C. elegans* microinjection assay, RNAi "fragments" of about 60 nucleotides with between 95 and 100% identity (to a nematode gene) were determined to cause excellent inhibition.

[00027] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. The nematode gene product can be inhibited with a RNAi molecule that is targeted to a portion or fragment of the exemplified polynucleotides; high homology (90-95%) or identity is also preferred, but not necessarily essential, for such applications.

[00028] The polynucleotide sequences identified in Appendix A and shown in the Sequence ID listing are from genes encoding nematode proteins having the functions

shown in Appendix 1. The genes exemplified herein are representative of particular classes of proteins which are preferred targets for disruption according to the subject invention. These classes of proteins include, for example, proteins involved in ribosome assembly; neurotransmitter receptors and ligands; electron transport proteins; metabolic pathway proteins; and protein and polynucleotide production, folding, and processing proteins.

[00029] Genetic regulatory sequences, such as promoters, enhancers, and terminators, can be used in genetic constructs to practice the subject invention. Such constructs themselves can also be used for nematode control. Various constructs can be used to achieve expression in specific plant tissues (by using root specific promoters, for example) and/or to target specific nematode tissues (by using targeting elements or adjacent targeting sequences, for example).

[00030] In a specific embodiment of the subject invention, plant cells, preferably root cells, are genetically modified to produce at least one RNAi that is designed to be taken up by nematodes during feeding to block expression (or the function of) of a target gene. As is known in the art, RNAi can target and reduce (and, in some cases, prevent) the translation of a specific gene product. RNAi can be used to reduce or prevent message translation in any tissue of the nematode because of its ability to cross tissue and cellular boundaries. Thus, RNAi that is contacted with a nematode by soaking, injection, or consumption of a food source will cross tissue and cellular boundaries. RNAi can also be used as an epigenetic factor to prevent the proliferation of subsequent generations of nematodes.

[00031] Nematode polynucleotide sequences disclosed herein demonstrate conserved nucleotide motifs among different nematode genera. Conserved nucleotide motifs strongly suggest that these sequences are associated with viability and/or parasitism and are functionally conserved and expressed in both *Meloidogyne incognita* (root-knot nematode) and *Globodera rostochiensis* and *Globodera pallida* (potato cyst nematodes). The use of these polynucleotides, and RNAi inhibitors thereof, is advantageous because such RNAi can be designed to have broad RNAi specificity and are thus useful for controlling a large number of plant parasitic nematodes *in planta*. Because the genes identified in this disclosure are associated with nematode survival

and/or parasitism, RNAi inhibition of these genes (arising from contacting nematodes with compositions comprising RNAi molecules) prevents and/or reduces parasitic nematode growth, development, and or parasitism.

[00032] Methods of the subject invention include the transformation of plant cells with genes or polynucleotides of the present invention, which can be used to produce nematode inhibitors or RNAi in the plants. In one embodiment, the transformed plant or plant tissue can express RNAi molecules encoded by the gene or polynucleotide sequence introduced into the plant. Other nematode inhibitors contemplated by the invention include antisense molecules specific to the polynucleotide sequences disclosed herein. The transformation of plants with genetic constructs disclosed herein can be accomplished using techniques well known to those skilled in the art and can involve modification of the gene(s) to optimize expression in the plant to be made resistant to nematode infection and infestation. Furthermore, it is known in the art that many tissues of the transgenic plants (such as the roots) can be targeted for transformation.

[00033] RNA-mediated interference (RNAi) of gene expression. Several aspects of root-knot nematode biology make classical genetic studies difficult with this organism. Since root-knot nematodes reproduce by obligatory mitotic parthenogenesis, the opportunity to perform genetic crosses is not available. Microinjection of RNAi can be used to manipulate gene expression in *C. elegans* (Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. [1998] "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" *Nature* 391:806-811). Microinjecting (into adult nematodes) RNAi can turn off specific genes in progeny worms complementary to the coding region of the genes. Moreover, gene inhibition occurs in progeny when RNAi is injected into the body cavity of the adult, indicating the ability of the RNAi to cross cellular boundaries. This RNAi injection method provides a molecular genetic tool that allows for analysis of gene function in root-knot nematodes.

[00034] RNAi can be taken up by *C. elegans* by simply soaking the nematodes in a solution RNAi. This results in targeted inhibition of gene expression in the nematode (Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto [1999] "RNAi screening with a non-redundant cDNA set" International Worm Meeting, Madison, WI, abstract 565). Nematodes fed *E. coli* expressing RNAi also demonstrate targeted and

heritable inhibition of gene expression (Sarkissian, M., H. Tabara and C. C. Mello [1999] "A mut-6 screen for RNAi deficient mutants" International Worm Meeting, Madison, WI, abstract 741; Timmons, L. and A. Fire [1998] "Specific interference by ingested dsRNA" *Nature* 395:854; WO 99/32619, hereby incorporated by reference in its entirety).

[00035] Accordingly, one aspect of the instant invention is directed to the control of nematodes comprising contacting nematodes with compositions comprising RNAi molecules specific to the nematode genes disclosed herein. The contacting step may include soaking the nematodes in a solution containing RNAi molecules, feeding nematodes RNAi molecules contained in microbes or plant cells upon which the nematode feeds, or injecting nematodes with RNAi. Nematodes can also be "contacted" and controlled by RNAi expressed in plant tissues that would be consumed, ingested, or frequented by nematodes.

[00036] The RNAi molecules provided to the nematodes may be specific to a single gene. A "cocktail" of RNAi molecules specific to various segments of a single gene can also be used. In addition, a "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) may be applied to the nematodes according to the subject invention.

[00037] In addition to RNAi uptake mediated by transgenic plants, nematodes can be directly transformed with RNAi constructs of cDNAs encoding secretory or other essential proteins to reduce expression of the corresponding gene. The transgenic animals can be assayed for inhibition of gene product using immunoassays or for reduced virulence on a host. Progeny of affected worms can also be assayed by similar methods.

[00038] Procedures that can be used for the preparation and injection of RNAi include those detailed by Fire *et al.*, (1998; <ftp://ciw1.ciwemb.edu>). Root-knot nematodes can be routinely monoxenically cultured on *Arabidopsis thaliana* roots growing on Gamborg's B-5/Gelrite® media. This nematode-host pathosystem is ideally suited for these microinjection experiments since limited root galling results in the parasitic stages (late J2 through adult females) developing outside of the root for easy accessibility for injecting. Another advantage is the parthenogenic reproduction of root-knot nematodes, which makes fertilization by males unnecessary for egg production. The RNAi can be injected into the body cavity of parasitic stages of root-knot nematodes

feeding on *A. thaliana* roots using microinjection. Control nematodes can be injected in parallel with only buffer or an unrelated RNAi. Injected nematodes can be monitored for egg production, and the eggs can be collected for the assays described below. Female root-knot nematodes will typically survive and lay more than 250 eggs following 1  $\mu$ l injection of buffer.

[00039] Alternatively, methods are available for microinjecting materials directly into the plant root cells upon which nematodes feed: giant cells or syncytial cells (Böckenhoff, A. and F.M.W. Grundler [1994] "Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*" *Parasitology* 109:249-254). This provides an excellent test system to screen RNAi molecules for efficacy by directly inhibiting growth and development of the nematode feeding upon the microinjected plant cell, or by reducing fecundity and the ability of said nematode to generate pathogenic or viable progeny.

[00040] There are a number of strategies that can be followed to assay for RNAi gene interference. Inhibition of gene expression by RNAi inhibits the accumulation of the corresponding secretory protein in the esophageal gland cells of transgenic J2 hatched from the eggs produced by the injected nematodes. In the first assay, polyclonal antibodies to the target gene product can be used in immunolocalization studies (Hussey, R. S. [1989] "Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species" *J. Nematol.* 21:392-398; Borgonie, G, E. van Driessche, C. D. Link, D. de Waele, and A. Coomans [1994] "Tissue treatment for whole mount internal lectin staining in the nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus*" *Histochemistry* 101:379-384) to monitor the synthesis of the target protein in the gland cells of progeny of the injected nematodes, or in any other nematode tissue that fails to express the essential targeted gene. Interference of endogenous gene activity by the RNAi eliminates binding of the antibodies to secretory granules in the glands, or any other target tissue, of the transgenic nematodes, and can be monitored by these *in situ* hybridization experiments. Control nematodes injected only with the injection buffer can be processed similar to the RNAi treated nematodes.



[00041] Another assay is designed to determine the effect of the RNAi on reducing the virulence of J2 progeny of the injected females. Egg masses from injected females can be transferred singly to *A. thaliana* plates to assess the ability of the transgenic J2 to infect roots. The J2 hatching from the eggs transferred to the plates can be monitored; after 25 days the number of galls with egg laying females can be recorded. The *A. thaliana* roots can also be stained with acid fuchsin to enumerate the number of nematodes in the roots. Egg masses from nematodes injected only with the injection buffer can be handled similarly and used as controls. The treatments can be replicated, and the root infection data can be analyzed statistically. These experiments can be used to assess the importance of the target genes in root-knot nematode's virulence or viability. By staining the J2 progeny of the injected females with the antibodies, it can be determined whether RNAi blocks expression of the targeted gene.

[00042] Additional uses of polynucleotides. The polynucleotide sequences exemplified herein can be used in a variety of ways. These polynucleotides can be used in assays for additional polynucleotides and additional homologous genes, and can be used in tracking the quantitative and temporal expression of parasitism genes in nematodes. These polynucleotides can be cloned into microbes for production and isolation of their gene products. Among the many uses of the isolated gene product is the development of additional inhibitors and modifiers. The protein products of the subject polynucleotides can also be used as diagnostic tools. For example, proteins encoded by the parasitism genes, as identified herein, can be used in large scale screenings for additional peptide inhibitors. The use of peptide phage display screening is one method that can be used in this regard. Thus, the subject invention also provides new biotechnological strategies for managing nematodes under sustainable agricultural conditions.

[00043] Antisense technologies can also be used for phytopathogenic nematode control. Antisense technology can be used to interfere with expression of the disclosed endogenous nematode genes. Antisense technology can also be used to alter the components of plants used as targets by the nematodes. For example, the transformation of a plant with the reverse complement of an endogenous gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and gene silencing

or inhibition of a target involved in the nematode infection process. Thus, the subject invention includes transgenic plants (which are preferably made nematode-resistant in this manner, and other organisms including microbes and phages) comprising RNAi or antisense molecules specific to any of the polynucleotides identified herein.

[00044] ~~Polynucleotide probes.~~ DNA possesses a fundamental property called base complementarity. In nature, DNA ordinarily exists in the form of pairs of anti-parallel strands, the bases on each strand projecting from that strand toward the opposite strand. The base adenine (A) on one strand will always be opposed to the base thymine (T) on the other strand, and the base guanine (G) will be opposed to the base cytosine (C). The bases are held in apposition by their ability to hydrogen bond in this specific way. Though each individual bond is relatively weak, the net effect of many adjacent hydrogen bonded bases, together with base stacking effects, is a stable joining of the two complementary strands. These bonds can be broken by treatments such as high pH or high temperature, and these conditions result in the dissociation, or "denaturation," of the two strands. If the DNA is then placed in conditions which make hydrogen bonding of the bases thermodynamically favorable, the DNA strands will anneal, or "hybridize," and reform the original double-stranded DNA. If carried out under appropriate conditions, this hybridization can be highly specific. That is, only strands with a high degree of base complementarity will be able to form stable double-stranded structures. The relationship of the specificity of hybridization to reaction conditions is well known. Thus, hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest.

[00045] The specifically exemplified polynucleotides of the subject invention can themselves be used as probes. Additional polynucleotide sequences can be added to the ends of (or internally in) the exemplified polynucleotide sequences so that polynucleotides that are longer than the exemplified polynucleotides can also be used as probes. Thus, isolated polynucleotides comprising one or more of the exemplified sequences are within the scope of the subject invention. Polynucleotides that have less nucleotides than the exemplified polynucleotides can also be used and are contemplated within the scope of the present invention. For example, for some purposes, it might be

useful to use a conserved sequence from an exemplified polynucleotide wherein the conserved sequence comprises a portion of an exemplified sequence. Thus, polynucleotides of the subject invention can be used to find additional, homologous (wholly or partially) genes.

[00046] Probes of the subject invention may be composed of DNA, RNA, or PNA (peptide nucleic acid). The probe will normally have at least about 10 bases, more usually at least about 17 bases, and may have about 100 bases or more. Longer probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probe sequence is designed to be at least substantially complementary to a portion of a gene encoding a protein of interest. The probe need not have perfect complementarity to the sequence to which it hybridizes. The probes may be labeled utilizing techniques that are well known to those skilled in this art.

[00047] One approach for the use of the subject invention as probes entails first identifying DNA segments that are homologous with the disclosed nucleotide sequences using, for example, Southern blot analysis of a gene bank. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new polynucleotides, and of the individual gene products expressed by a given polynucleotide. Such an analysis provides a rapid method for identifying commercially valuable compositions.

[00048] One hybridization procedure useful according to the subject invention typically includes the initial steps of isolating the DNA sample of interest and purifying it chemically. Either lysed nematodes or total fractionated nucleic acid isolated from nematodes can be used. Cells can be treated using known techniques to liberate their DNA (and/or RNA). The DNA sample can be cut into pieces with an appropriate restriction enzyme. The pieces can be separated by size through electrophoresis in a gel, usually agarose or acrylamide. The pieces of interest can be transferred to an immobilizing membrane.

[00049] The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

[00050] The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or very similar. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred.

[00051] In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. In addition, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[00052] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[00053] As used herein "moderate to high stringency" conditions for hybridization refers to conditions that achieve the same, or about the same, degree of specificity of hybridization as the conditions "as described herein." Examples of moderate to high stringency conditions are provided herein. Specifically, hybridization of immobilized DNA on Southern blots with  $^{32}\text{P}$ -labeled gene-specific probes was performed using standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that

allowed for detection of target sequences with homology to sequences exemplified herein. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25 ° C below the melting temperature ( $T_m$ ) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula from Beltz *et al.* (1983):

[00054]  $T_m = 81.5^\circ\text{C} + 16.6 \log[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs}.$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20^\circ\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[00055] For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature ( $T_m$ ) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA.  $T_m$  for oligonucleotide probes was determined by the following formula from Suggs *et al.* (1981):

[00056]  $T_m (^\circ\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$

[00057] Washes were typically carried out as follows:

- [00058] (1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).
- [00059] (2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[00060] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment of greater than about 70 or so bases in length, the following conditions can be used:

Low:	1 or 2X SSPE, room temperature
Low:	1 or 2X SSPE, 42°C
Moderate:	0.2X or 1X SSPE, 65°C
High:	0.1X SSPE, 65°C.

[00061] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch

can be tolerated. Therefore, polynucleotide sequences of the subject invention include mutations (both single and multiple), deletions, and insertions in the described sequences, and combinations thereof, wherein said mutations, insertions, and deletions permit formation of stable hybrids with a target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence using standard methods known in the art. Other methods may become known in the future.

[00062] The mutational, insertional, and deletional variants of the polynucleotide sequences of the invention can be used in the same manner as the exemplified polynucleotide sequences so long as the variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide similarity that is sufficient to enable the variant polynucleotide to function in the same capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[00063] PCR technology. Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159; Saiki *et al.*, 1985). PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA fragment produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a

few hours. By using a thermostable DNA polymerase such as *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes that can be used are known to those skilled in the art.

[00064] The polynucleotide sequences of the subject invention (and portions thereof such as conserved regions and portions that serve to distinguish these sequences from previously-known sequences) can be used as, and/or used in the design of, primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5' end) of the exemplified polynucleotides can be used in this manner. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

[00065] The polynucleotide sequences of the instant invention may be "operably linked" to regulatory sequences such as promoters and enhancers. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is "operably linked" to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is "operably linked" to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is "operably linked" to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00066] Polynucleotides and proteins. Polynucleotides of the subject invention can be defined according to several parameters. One characteristic is the biological activity of the protein products as identified herein. The proteins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain

exemplified probes and primers. Additional primers and probes can readily be constructed by those skilled in the art such that alternate polynucleotide sequences encoding the same amino acid sequences can be used to identify and/or characterize additional genes. The proteins of the subject invention can also be identified based on their immunoreactivity with certain antibodies.

[00067] The polynucleotides and proteins of the subject invention include portions, fragments, variants, and mutants of the full-length sequences as well as fusions and chimerics, so long as the encoded protein retains the characteristic biological activity of the proteins identified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences that encode the same proteins or which encode equivalent proteins having equivalent biological activity. As used herein, the term "equivalent proteins" refers to proteins having the same or essentially the same biological activity as the exemplified proteins.

[00068] It will be apparent to a person skilled in this art that genes within the scope of the subject invention can be identified and obtained through several means. The specific genes exemplified herein may be obtained from root-knot nematodes. Genes, or portions or variants thereof, may also be artificially synthesized by, for example, a gene synthesizer.

[00069] Variations of genes may be readily constructed using standard techniques such as site-directed mutagenesis and other methods of making point mutations and by DNA shuffling, for example. In addition, gene and protein fragments can be made using commercially available exonucleases, endonucleases, and proteases according to standard procedures. For example, enzymes such as *Bal31* can be used to systematically cut off nucleotides from the ends of genes. In addition, genes that encode fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins. Of course, molecular techniques for cloning polynucleotides and producing gene constructs of interest are also well known in the art. *In vitro* evaluation techniques, such as MAXYGEN's "Molecular Breeding" can also be applied to practice the subject invention.

[00070] Other molecular techniques can also be applied using the teachings provided herein. For example, antibodies raised against proteins encoded by



polynucleotides disclosed herein can be used to identify and isolate proteins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the proteins that are conserved and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to proteins encoded by polynucleotides disclosed herein, or to equivalent proteins, can readily be prepared using standard procedures known in the art. The genes that encode these proteins can be obtained from various organisms.

[00071] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences encoded by the polynucleotide sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding proteins having the same, or essentially the same, amino acid sequence. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences that have amino acid substitutions, deletions, additions, or insertions that do not materially affect biological activity. Fragments retaining the characteristic biological activity are also included in this definition.

[00072] A further method for identifying genes and polynucleotides (and the proteins encoded thereby) of the subject invention is through the use of oligonucleotide probes. Probes provide a rapid method for identifying genes of the subject invention. The nucleotide segments that are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[00073] The subject invention comprises variant or equivalent proteins (and nucleotide sequences coding for equivalent proteins or for inhibitors of the genes encoding such proteins) having the same or similar biological activity of inhibitors or proteins encoded by the exemplified polynucleotides. Equivalent proteins will have amino acid similarity with an exemplified protein (or peptide). The amino acid and/or nucleotide identity will typically be greater than 60%. Preferably, the identity will be greater than 75%. More preferably, the identity will be greater than 80%, and even more preferably greater than 90%. Most preferably, the identity will be greater than 95%. RNAi molecules will also have corresponding identities in these preferred ranges. These

identities are as determined using standard alignment techniques for determining amino acid and/or nucleotide identity. The identity/similarity will be highest in critical regions of the protein or gene including those regions that account for biological activity or that are involved in the determination of three-dimensional configuration that is ultimately responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Below is a list of examples of amino acids belonging to various classes

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

[00074] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not detract from the ability to manage nematode-caused diseases.

[00075] An "isolated" or "substantially pure" nucleic acid molecule or polynucleotide is a polynucleotide that is substantially separated from other polynucleotide sequences which naturally accompany a nucleic acid molecule. The term embraces a polynucleotide sequence which was removed from its naturally occurring environment by the hand of man. This includes recombinant or cloned DNA isolates,

chemically synthesized analogues and analogues biologically synthesized by heterologous systems. An "isolated" or "purified" protein, likewise, is a protein removed from its naturally occurring environment.

[00076] Recombinant hosts. The genes, antisense, and RNAi polynucleotides within the scope of the present invention can be introduced into a wide variety of microbial or plant hosts. Plant cells can be transformed (made recombinant) in this manner. Microbes, for example, can also be used in the application of RNAi molecules of the subject invention in view of the fact that microbes are a food source for nematodes.

[00077] There are many methods for introducing a heterologous gene or polynucleotide into a host cell or cells under conditions that allow for stable maintenance and expression of the gene or polynucleotide. These methods are well known to those skilled in the art. Synthetic genes, such as, for example, those genes modified to enhance expression in a heterologous host (such as by preferred codon usage or by the use of adjoining, downstream, or upstream enhancers) that are functionally equivalent to the genes (and which encode equivalent proteins) can also be used to transform hosts. Methods for the production of synthetic genes are known in the art.

[00078] Where the gene or polynucleotide of interest is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes are preferred. Certain microorganism hosts are known to occupy the phytosphere, phylloplane, phyllosphere, rhizosphere, and/or rhizoplane of one or more crops of interest. These microorganisms can be selected so as to be capable of successfully competing in the particular environment (crop and other habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing a polypeptide of interest, and, desirably, provide for improved protection of the protein/peptide from environmental degradation and inactivation.

[00079] A large number of microorganisms is known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*,

*Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are the pigmented microorganisms.

[00080] Methods of the subject invention also include the transformation of plants or plant tissue with genes which encode the RNAi molecules of the present invention. In one embodiment, the transformed plant or plant tissue expresses antisense RNA and/or RNAi. Transformation of cells can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

[00081] Additional methods and formulations for control of pests. Control of nematode pests using the RNAi molecules of the instant invention can be accomplished by a variety of additional methods that would be apparent to those skilled in the art having the benefit of the subject disclosure. A "cocktail" of two or more RNAi molecules can be used to disrupt one or more of the genes identified herein. The "cocktail" of RNAi molecules may be specific to segments of a single gene or the entire gene. A "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) is also encompassed by the instant invention. In another embodiment of the instant invention, the disclosed RNAi molecules, cocktails, and/or multigene cocktails thereof, may be used in conjunction with other known nematode control agents and methodologies. Such cocktails can be used to combat the development of resistance by nematodes to a certain inhibitor or inhibitors.

[00082] Compositions of the subject invention which comprise RNAi molecules and carriers can be applied, themselves, directly or indirectly, to locations frequented by, or expected to be frequented by, nematodes. Microbial hosts which were transformed with polynucleotides that encode RNAi molecules, express said RNAi molecules, and which colonize roots (e.g., *Pseudomonas*, *Bacillus*, and other genera) can be applied to the sites of the pest, where they will proliferate and be ingested. The result is control of the pest. Thus, methods of the subject invention include, for example, the application of recombinant microbes to the pests (or their locations). The recombinant microbes may also be transformed with more than one RNAi molecule thereby delivering a "cocktail" of RNAi molecules to the nematode pests. A carrier may be any substance suitable for

delivering the RNAi molecules to the nematode. Acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

[00083] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[00084] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1— Production of Hairy Roots for RNAi Testing

[00085] A hairy root assay system was developed for testing the anti-nematode activity of RNAi molecules.

[00086] *Agrobacterium rhizogenes*: Several *Agrobacterium rhizogenes* strains produce hairy roots on a variety of plant species. *A. rhizogenes* strains, A4, 15834, 8196 and LBA4404 demonstrate hairy root development on tomato and sugar beet, with A4 being the most efficient. The *A. rhizogenes* strain K599 demonstrated very efficient formation on transgenic soybean hairy roots and was also effective on sugar beet and *Arabidopsis*. However, strain K599 failed to produce hairy roots on tomato tissues possibly due to hyper-virulence.

[00087] Hairy root production: Transgenic hairy roots were identified by stable GUS expression in tomato, sugar beet, soybean and *Arabidopsis*. The construct pAKK1401 (pNOS / NPT-II / tNOS // pSU / GUS / tNOS) was used to produce hairy roots when transformed into *A. rhizogenes* strains A4 or K599. Transgenic roots were identified by GUS expression.

#### Example 2— Protocol for Electro-competent *Agrobacterium* and Electroporation

[00088] Electro-competent *Agrobacterium* Protocol:

- [00089] 1. Grow *Agrobacterium* overnight in 5 mls LB + antibiotics at 30°C on shaker (for *Agrobacterium rhizogenes* strain K599 no antibiotics are needed).
- [00090] 2. Use the 5 mls of overnight culture to inoculate 500 mls LB + antibiotics at 30°C on shaker. Grow overnight.
- [00091] 3. Add liquid culture in eight 50 ml polypropylene orange cap tubes.
- [00092] 4. Centrifuge 10 min., 4000 rpm, 4°C.
- [00093] 5. Resuspend cells in each tube with 20 mls 10% glycerol (on ice)
- [00094] 6. Centrifuge 10 min., 4000 rpm, 4°C.
- [00095] 7. Resuspend cells in each tube with 10 mls 10% glycerol (on ice).
- [00096] 8. Centrifuge 10 min., 4000 rpm, 4°C.
- [00097] 9. Resuspend cells in each tube with 2 mls 10% glycerol (on ice).
- [00098] 10. Aliquot 50 µl into cold Eppendorf tube and place onto dry ice.
- [00099] 11. Store electro-competent cells at -80°C. These cells can be used for up to two years.

[000100] Electroporations:

- [000101] 1. Add 1 µl to 5 µl of DNA (resuspended in H<sub>2</sub>O and not TE or other buffer) to 50 µl of *Agrobacterium* electrocompetent cells and mix.
- [000102] 2. Transfer 20 µl of DNA/*Agrobacterium* mix to cuvette.
- [000103] 3. Electroporate:  
25µF, 400 Ω resistance, 2.5 volts (0.2cm cuvette) or 1.8 volts (0.1cm cuvette for BioRad electroporator. 330 µF, 4000 kΩ, low w, fast charge rate for BRL Electroporator.
- [000104] 4. Add 1ml of LB and transfer to Eppendorf tube.
- [000105] 5. Shake at 30°C for 2 hours.
- [000106] 6. Centrifuge down cells (2 min. 14 krpm).
- [000107] 7. Plate all onto LB + antibiotics (most *Agrobacterium* strains are naturally streptomycin resistant).

Example 3 – Protocol for Production of Transgenic Hairy Roots on Soybean

[000108] Seed Sterilization. Rinse the soybean seed with 70% ETOH for 2-5 min. Remove and add 20% Clorox and shake for 20-25 min. Rinse 3X with sterile water. Plate the seed, 5 seed per plate, onto  $\frac{1}{2}$  MSB5 + 2% sucrose + 0.2% gel (referred to as  $\frac{1}{2}$  MSB5). Place seed into chamber at 25C, 16/8 photoperiod for 5-7 day (depending on genotype) germination period. After 1 week seedlings can be placed into cold room for longer storage if necessary (not to exceed 2 weeks).

[000109] Agrobacterium Preparation. For Agrobacterium rhizogenes strain K599, take a small sample from frozen glycerol into 25-50 ml of NZYM media with 50 mg/L kanamycin in a 125-250 ml Erlenmeyer flask. Place onto shaker at 28-30 °C for 16 - 20 hours. Pour sample into centrifuge tube and centrifuge the bacterium at 4000 rpm for 10 min. Pour off supernatant and re-suspend the pellet with an equal volume of liquid  $\frac{1}{2}$  MSB5 + 200  $\mu$ M acetosyringone. Use pipette to re-suspend the pellet and homogenize the sample (remove all clumps). To determine O.D., prepare a 1:10 dilution by putting 900  $\mu$ l  $\frac{1}{2}$  MSB5 into cuvette and add 100  $\mu$ l of bacterial sample. Determine the O.D.<sub>660</sub> and calculate the volume needed to adjust (dilute) OD to approximately 0.2 for inoculation. Check final O.D.

[000110] Explant Preparation and inoculation. Place a sterile filter paper onto plates of  $\frac{1}{2}$  MSB5. Cut soybean cotyledons just above the shoot apex and place onto plate. Lightly scar the cotyledon's abaxial surface (flat side, upper surface that reaches toward sun) with a scalpel blade. Cut each cotyledon transversely into 2-3 pieces (no smaller than 1 cm). Add approximately 10 ml of prepared bacterial solution to each plate and allow cotyledons to incubate for 1 hr. Remove the bacteria using a vacuum aspirator fitted with sterile pipette tip, ensure that there is no standing liquid. Orient all explants with abaxial surface up and wrap plates for a 3 day co-culture, 25°C in light (16/8 photoperiod).

[000111] Hairy root selection and maintenance. After 3 day co-culture, wash explants with liquid  $\frac{1}{2}$  MSB5 + 500 mg/L carbenicillin. Transfer the explants abaxial side up to selection media,  $\frac{1}{2}$  MSB5 supplemented with 500 mg/L carbenicillin and 200 mg/L kanamycin. Roots should develop in approximately 2-3 weeks. The roots will form primarily from the cut vascular bundles with other roots developing from the small cuts on cotyledon surface. Remove roots (>1cm in length) and place onto replica media with

transfers to fresh media every 2 weeks to prevent *Agrobacterium* overgrowth. After 6-8 weeks on selection the roots can be moved to media without kanamycin, however carbenicillin must remain in media for several months for continued suppression of *Agrobacterium*. At this stage roots can be used for testing RNAi for nematode control. Sterilized nematodes can be added and observed for RNAi affects.

#### Example 4 – Testing of RNAi for Plant Parasitic Nematode Control.

[000112] Various types of nematodes can be used in appropriate bioassays. For example, *Caenorhabditis elegans*, a bacterial feeding nematode, and plant parasitic nematodes can be used for bioassay purposes. Examples of plant parasitic nematodes include a migratory endo-parasite, *Pratylenchus scribneri* (lesion), and two sedentary endo-parasites, *Meloidogyne javanica* (root-knot) and *Heterodera schachtii* (cyst).

[000113] *C. elegans*: RNAi vectors can be tested through expression of the RNAi in *E. coli*. *C. elegans* are fed *E. coli* and assayed for their growth by measuring growth of nematodes, production of eggs and viability of offspring. Another approach is to inject dsRNA directly into living nematodes. Finally, soaking nematodes in a solution of *in vitro*-prepared RNAi can quickly establish efficacy of treatment.

[000114] *P. scribneri*: The *P. scribneri in vitro* feeding assay uses a corn root exudate (CRE) as a feeding stimulus and both the red dye Amaranth or potassium, arsenate as feeding indicators. Feeding is confirmed after seven days by the presence of red stained intestinal cells in live worms exposed to the Amaranth or death of worms exposed to arsenate. This bioassay is used to test soluble toxins or RNAi. *P. scribneri* has also been cultured on wild type roots of corn, rice and *Arabidopsis*, and on A. rhizogenes-induced hairy roots of sugar beet and tomato. *P. scribneri* is very valuable in evaluating transgenic hairy roots because of the non-specific feeding of these worms.

[000115] *M. javanica*: Nematode eggs are sterilized using bleach and are used to inoculate hairy roots expressing RNAi. Nematodes are assessed for their growth by measuring knots, egg masses or production of viable eggs. An alternative approach is to microinject dsRNA directly into root feeding sites or into living female nematodes.

[000116] *H. schachtii*: Cultures of this nematode were maintained on sugar beets. Nematodes eggs are sterilized using bleach and used to inoculate hairy roots



expressing RNAi. Nematodes can be assessed for their growth by measuring knots, egg masses or production of viable eggs.

#### Example 5 – Plant Expression Vectors for RNAi

[000117] Modular Binary Construct System (MBCS): An important aspect of the subject disclosure is the Modular Binary Construct System. The MBCS eases the burden of construct development by creating modular pieces of DNA that can be easily added, removed, or replaced with the use of low frequency cutting restriction enzymes (8-base cutters). These constructs are useful for delivery of a variety of genes to plant cells and is not limited to the delivery of RNAi genes. To develop this system, a series of six, 8-base cutter restriction enzyme sites was placed between the left and right Ti borders of a previously created *kan<sup>R</sup>/tet<sup>R</sup>* binary plasmid (Figure 1). The production of both *kan<sup>R</sup>* and *tet<sup>R</sup>* MCBS aids the testing of constructs using different strains of *Agrobacterium rhizogenes* in different plant species. In addition to the MBCS, a series of shuttle vectors were created that aid in the cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites (Figure 2). With six 8-base cutter sites, each site is, preferably, reserved for a particular function (Figures 3 and 4). Because of the close proximity of the *Pme* I and *Sgf* I sites to the left and right border of the binary vector, these sites are, preferably, reserved for gene tagging and enhancer trap experiments. The *Not* I site is, preferably, reserved for plant selectable markers (Figure 5). The *Pac* I site is reserved, preferably, for Plant Scorable Markers (Figure 6). The *Asc* I site is, preferably, reserved for RNAi experiments (Figures 7 and 8), while the *Sbf* I site is, preferably, reserved for anti-nematode proteins. The restriction sites that are denoted in the Figures are, preferably, reserved for the denoted insertions; however, the MCBS binary and shuttle vectors do not require the restriction sites to contain these suggested inserts.

[000118] Plant Selectable Markers for MBCS: To further develop the MBCS, a series of plant selectable markers were added to the MBCS (Figure 5). Plant selectable markers that were added to the MBCS include: *pNOS/NPT-II/tNOS* (*kan<sup>R</sup>*), *pNOS/Bar/tNOS* (*basta<sup>R</sup>* for dicots), *pUBI/Intron-Bar/tNOS* (*basta<sup>R</sup>* for monocots), and *pUBI/Intron-PMI/tNOS* (mannitol isomerase<sup>R</sup>).

[000119] Reporter Genes for MBCS: Four exemplary reporter genes are used in the MBCS are provided in Figure 6 and Appendix 2. GUS, a nuclear localized GUS, GEP, and the anthocyanin transcriptional activator *papIC* genes into the MBCS.

[000120] Promoters for MBCS: We cloned several useful constitutive and nematode-inducible promoters (Figures 6, 7 and Appendix 2). Constitutive promoters include the SuperUbiquitin promoter from pine (pSU) and two promoter regions from the Strawberry Banding Vein virus (pSBV<sub>1</sub> and pSBV<sub>2</sub>). Seven nematode-inducible promoters from *Arabidopsis* were also been cloned.

[000121] The following Scorable marker clones have been constructed and placed in the MBCS, NPT-II binary vector (pNOS/NPT-II/tNOS):

Intron/GUS/tNos	Intron/NLS-GUS/tNOS	Intron/GFP/tNOS
pSU/Intron/GUS/tNOS	pSU/Intron/NLS-GUS/tNOS	pSU/Intron/GFP/tNOS
pSBV <sub>1</sub> /Intron/GUS/tNOS	pSBV <sub>1</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>1</sub> /Intron/GFP/tNOS
pSBV <sub>2</sub> /Intron/GUS/tNOS	pSBV <sub>2</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>2</sub> /Intron/GFP/tNOS
pKT/Intron/GFP/tNOS		
pKA/Intron/GFP/tNOS		

#### Example 6 – Control of Plant parasitic nematodes using RNAi in planta

[000122] Production of RNAi Vector. The RNAi shuttle vector to be used is adapted from the Modular Binary Construct System (MBCS - See Example 5). RNAi shuttle vectors preferably comprise a promoter, intron, antisense RNAi, stuffer fragment, sense RNAi, and terminator (See Figures 7 and 8 and Appendix 2 for more details). The plant promoter can be constitutive, tissue-specific or nematode-inducible. The intron is necessary to eliminate expression in *Agrobacterium*.

[000123] The anti-sense and sense RNAi molecules comprise nematode-specific sequences and are disclosed herein. These genes are associated with pathogenesis, growth, or other cellular function in nematodes. An exemplary group of RNAi sequences for use in plant/nematode control may be based upon:

[000124] 1. Genes specific for nematode esophageal gland cells.

[000125] 2. Genes specific for plant parasitic nematodes but not other free living nematodes.

- [000126] 3. Genes common to all plant parasitic nematodes.
- [000127] 4. Genes common to all nematodes (nematode-specific).
- [000128] 5. Genes specific for important tissues or cell types.
- [000129] 6. Genes from large gene families.
- [000130] 7. Genes involved in nematode signal transduction or other cellular pathways.

[000131] Appropriate RNAi constructs allow for the formation of dsRNA molecules (the sense and antisense strands join to form the dsRNA). The terminator sequence adds a poly-A tail for transcriptional termination. The RNAi shuttle vector can then be subcloned into the MBCS and transformed into *Agrobacterium rhizogenes*.

[000132] Plant Transformation with RNAi Vectors. An exemplary transformation system for generating hairy roots using *Agrobacterium rhizogenes* is provided below. The RNAi vector once introduced into the MBCS can subsequently (as a binary vector) be transformed in *A. rhizogenes* using, for example, the electroporation protocol of Example 2. Once the *A. rhizogenes* is confirmed to contain the plasmid, it is then used in generating hairy roots (See Example 3). Using this protocol transgenic hairy roots expressing RNAi are isolated, cultured and tested.

[000133] Testing of RNAi Vector for Nematode or Plant Pathogen Resistance. RNAi expressing hairy roots can be inoculated with sterilized nematodes. Infested hairy roots can be observed and the effect on nematodes determined. An alternative approach involves the microinjection of RNAi directly into root feeding sites (giant-cells for root-knot nematode, and syncytia for cyst nematodes) or into living female nematodes.

#### Example 7 – Insertion of Genes Into Plants

[000134] One aspect of the subject invention is the transformation of plants with genes encoding proteins of the present invention. Transformation of plants as described herein can be used to improve the resistance of these plants to attack by the target pest.

[000135] Genes, polynucleotides, and/or RNAi molecules as disclosed or suggested herein can be inserted into plant cells using a variety of techniques which are

well known in the art. For example, a large number of cloning vectors, for example, pBR322, pUC series, M13mp series, pACYC184, pMON, *etc.*, are available for preparation for the insertion of foreign genes into higher plants via injection, biolistics (microparticle bombardment), *Agrobacterium tumefaciens*, or *Agrobacterium rhizogenes*-mediated transformation, or electroporation as well as other possible methods. Once the inserted DNA has been integrated into the genome, the genetically modified-cell(s) can be screened via a vector carried-selectable marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or bialophos, *inter alia*. The transformed cell will be regenerated into a morphologically normal plant. The transgene(s) in the transgenic plant is relatively stable and can be inherited by progeny plants.

[000136] If a transformation event involves a germ line cell, then the inserted DNA an corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

[000137] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

We claim:

1. An RNAi molecule, optionally comprising a linker, wherein at least one strand of said RNAi is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 139.

2. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
1.

3. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
2.

4. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
3.

5. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
4.

6. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
5.

7. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
6.

8. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
7.

9. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
8.

10. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
9.

10. 11. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
12.
11. 12. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
11.
12. 13. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
12.
13. 14. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
13.
14. 15. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
14.
15. 16. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
15.
16. 17. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
16.
17. 18. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
17.
18. 19. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
18.
19. 20. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
19.
20. 21. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
20.

21. 22. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 21.
22. 23. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 22.
23. 24. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 23.
24. 25. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 24.
25. 26. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 25.
26. 27. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 26.
27. 28. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 27.
28. 29. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 28.
29. 30. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 29.
30. 31. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 30.
31. 32. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 31.

34

32. 33. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
33. 34. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
34. 35. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
35. 36. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
36. 37. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
37. 38. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
38. 39. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
39. 40. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
40. 41. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
41. 42. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
42. 43. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:



44. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
43.
45. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
44.
46. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
45.
47. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
46.
48. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
47.
49. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
48.
50. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
49.
51. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
50.
52. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
51.
53. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
52.
54. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
53.

54. 55. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
55. 56. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
56. 57. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
57. 58. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
58. 59. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
59. 60. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
60. 61. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
61. 62. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
62. 63. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
63. 64. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
64. 65. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

65. 66. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
66. 67. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
67. 68. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
68. 69. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
69. 70. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
70. 71. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
71. 72. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
72. 73. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
73. 74. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
74. 75. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
75. 76. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

76. 77. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
77. 78. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
78. 79. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
79. 80. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
80. 81. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
81. 82. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
82. 83. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
83. 84. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
84. 85. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
85. 86. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
86. 87. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

87. 88. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
87.
88. 89. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
88.
89. 90. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
89.
90. 91. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
90.
91. 92. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
91.
92. 93. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
92.
93. 94. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
93.
94. 95. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
94.
95. 96. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
95.
96. 97. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
96.
97. 98. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
97.

99. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 98.
100. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 99.
101. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 100.
102. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 101.
103. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 102.
104. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 103.
105. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 104.
106. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 105.
107. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 106.
108. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 107.
109. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 108.

110. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 109.

111. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 110.

112. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 111.

113. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 112.

114. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 113.

115. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 114.

116. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 115.

117. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 116.

118. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 117.

119. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 118.

120. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 119.

121. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 120.

122. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 121.

123. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 122.

124. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 123.

125. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 124.

126. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 125.

127. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 126.

128. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 127.

129. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 128.

130. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 129.

131. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 130.



132. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 131.

133. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 132.

134. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 133.

135. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 134.

136. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 135.

137. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 136.

138. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 137.

139. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 138.

140. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 139.

141. A transgenic plant or transgenic plant tissue comprising an RNAi molecule according to any of the preceding claims.

142. A method of disrupting cellular processes in a nematode comprising the steps of:
- (a) providing a composition comprising a compound according to any of the preceding claims; and
  - (b) contacting a nematode with said composition.

143. An isolated promoter comprising the following nucleotide sequence:

aacagcccaagataaca gaaaagtcaaagggtgttcgaaa  
gaccacttgtgactaaggatcattt catccataattatctggtagca  
cagactcatgataaetgcgaggaacacaagttctttacagtcgattc  
aaagacactttctctttacggtttcattgaaggagccgaccagaat  
atgtcagagaagcttttactgtgggttaatttcattaatctatcca  
ggtgaaaacctcaaggagatctctcttctccaaaagacctctacag  
ggcaatcaaaaactacagaaccagagtttgtagtgcacagagtagac  
caatctacctgagaatcacgagtaccttcttagagtgggaaaatgat  
gacatccttattccataccactgga ttgaggtaggactatccaatgg  
aaaaattccatgggacaagtcatat aagaagaccgcaacagtcgagt  
atcttccagagataactgcactcagacctaaggataaaagcagta  
tataatcagtgtaactaagatcttcgcagattcaaagaagaagcttaa  
ctatgctgatgacaagataattctaataagcaattattcagaattaa  
tcaaggagaaagaattaataactcttctcagaatatgaagcccgttt  
acaagtggtccagctagctatcactgaaaagacagcaagacaatggtg  
tctcgatgcaccagaaccacatctt tgcagcagatgtgaagcagcca  
gagtggtccacaagacgcactcagaaaaggcatcttctaccgacaca  
gaaaaagacaaccacagctcatcatccaacatgtagactgtcgttat  
gcgtcggctgaagataagactgacctcaggccagcactaaagaagaa  
ataatgcaagtggtccttagctccactttagctttaataattatgttt  
cattattattctctgcttttgctctctatataaagagcttgtatattt  
catttgaaggcagaggcgaacacacacacagaacctccctgcttaca  
aaccatgtattgttagctaaacctcttaggag .

144. An isolated promoter comprising the following nucleotide sequence:

tggtggggacaatggatccggtctgcgtagcaacaaggctg  
aaaaagattaaacagaaacctgtgatcattagcgttgaccaccacc  
aaaacctcctgagccaccaagcctccagagcctgaaaaaccaaagc  
ctccaccagcacctgaaccaccaagcatgtatgcaagccaccttac  
tgcaacagttgtgatgtgtgtctgttactacctatgaaagtggaag  
cggctgcaccattctttgagtcataatatcgcgtagcatagccttcac  
gttaagtccctgtatttagccaataactaattcatcatgttctcatgct  
tttttggtttatttctttttctcaaatatgaatctctgttgtttgtcc  
ctcccctgtttataattagtcgcttctttgacacaagaagtctcatg  
agttcatgctaagaaaaataaaagttcaaattaaaacaccaaatgtt  
tgattaatttccataaaacctgtgaagcagaaagttagtcattgttgac  
ctgaacagagccttaggaagtccttgaaggacatatcttcaagtgtta  
ttgggtcgtagcactcttaggcccatctaacttcattgagccatttaa  
attatgcaaaacaagaaatgagacatatggaacattagggttctta  
caggaaaaaataggaaaaagcagggacaactaaacaaaaattcagaa  
acaagaggcaagtggacgaccacggcgtaagatcaacatgtggtgat  
gtgcatgagaccaagaccattttttctcgttcttcaacgcacacttg  
gtctttttcttatgtttgttgcatctctttattagggcagaccctctct  
cttttttaataggatagtaaaaaatatatgattttattttgttgaaa  
cattttgagttaaaacctaaacttatagtaagcatttgtagagtga  
tttctctatagcatctatcaacatgaccttaacccaaaaaatatt  
gatgaaactactttaagtagtaaaacctaaagcaattaaaatttctt  
ttaaattagtagtttgtgtaaatttaattgacatgattgcgtcgaaag  
aaatcaaaacagttatatcgtagaacttaggagaatgttttatatcgt  
gtttcaacacatgattgctagcatatgtgtagggtgtcgtagacgtta  
cataacaatcatcactcgtaaatatcaaagtgggttctgagagaaac  
aaagggttatgattttcccaactgcactagttgtgtattgtttcttt  
cacacgtatgcttctgagttctgcccaaagtggaaattaaagcagag  
ttgggagagatcataatttattaggggttcgttatgctcaagtcatga  
cgtaaaatgaaaatttggtttttattctttcaccaacacaaagaatag  
ctagttatctctttttttatatataacaattcatgaagttgatcagc  
tttatcacatcatccaatcgaattgctaattctagagatggaaatat  
caggatagagccaataagatatcaaattcaatggaccattttctctcc  
atgtgctaattcatacaatctgtttttgtctgctttatttgatgatg  
atgctgagcgttttttaagtgtgaactaagatctagctaaccaaaaca  
aagatgggtctcttctgtctttgtcggtataagagcaagagagtggttt  
gattcaatttttaaaattctaaataaaaactccaaccgtgaatccagc  
catgaaactcttttttagaaaatcctttttttataacaaataattcttc  
tgcttcttcttcttcttctgtttattttcaccttttttggtttctttag  
ctcagaaaaagccattctttttttctattcttgtttatttttaatca  
tactgtgcgtttctacaaagttgttcttcttcttcttcaactctctc  
actcacagtcacagagatctgtttctttttctttttttgctttcactc  
ttctcttccagt.

145. An isolated promoter comprising the following nucleotide sequence:

.agcaaagcaagaacaccagagaagaagaaaagcactacaga  
gaaaaatgtgagcttaagcgtctccaacaacacttctctgggagtc  
taaaggatgctgcaaaaagccttggtggtgagacttccgcataattc  
caagcatgggtttatTTTTgttagcacacaaactatctgaccctcga  
cttggattttctctgcagtttgtccaactacattgaaacggatatg  
caggcaacatgggatcatgaggtggccatctcgtaagattaacaaag  
tgaaacaggtcactaaggaaaatacagacgggtactggactcggccaa  
ggtgtagaaggaggactaaagtccgactcagcaactggcgaattcat  
tgcagtttagaccttttattcaagaaattgatacccaaaagggctctgt  
cgtctcttgataatgatgcacatgcaagaagaagtcaggaggatatg  
cctgacgatacttcattcaagctccaggaagctaaatctgtcgcacaa  
tgccattaagtttagaggaggatacaaccatgaatcaagcaagaccag  
gtaagaacttctctatccataaaccatagatggagcgattagaatct  
taatccattttcagtttttgcaggatcattcatggagggttaatgcta  
gtggtcagccatgggcttggtatggccaaagagtcctggcttgaatggc  
agtgaaggaataaagagcgtttgcaacttaagctctgtggaaatttc  
agatggaatggatccaacaatccgatgcagtggcagttattgttgaa  
ctaaccaatccatgtcatgcagcatatcagattcatcaaatggctca  
ggcgcagttctgcgtggaagctcatctacttccatggaagattggaa  
ccaaatgagaaccacacagtaataagcagcgagagtggtcaacaa  
cgctgatcgtaaaggccagttatagagaagacactgtacgtttcaag  
ttcgagccatcagttgggtgtcctcagctctacaaagaagttggaaa  
acgttttaaaactgcaggacgggtcgtttcagctgaagtacttggatg  
atgaagaagaatgggtgatgctggttacagattctgatctccaagaa  
tgtttggagatattacatggtatgggaaaacactcgggtgaagtttct  
cgttcgtgattttgtctgcccctctaggtagttctgggtggcagtaatg  
gttatcttggaaacagggttatgacgtcgtaagacatagacacacaca  
gttatgtattcccagtgaaagaatgttggtttatttctctagatatta  
gtatgcttataaataggcatgaaggagaaagacaattttgggtatagt  
ggagttcagcagaaaaatgtatatgtttttcgttttatatgaatcag  
agaataaaaagttggatgttatatctacgttgctaattgtgtacctgc  
tcacccatctttcatataagaaaaagagaacacttttagttatccctg  
tgatgcagaatcgattctttgttatctctccattcctgtggaaacc  
aacaagtcaactaaatttcgggtttaattgggtgggtttttaagtcaa  
cgaggacttgatttttagttgggcttgggcctataattgtgttcatca  
ttgggttttttcccccttatcagtttaacgtccatatccatatcttt  
ttcttttttaacggcaaaagttcatatccatatcttatgatgtgcct  
aaaagagggagaagatgcgaagacagaattttcatatttgaaagggt  
tcgatatcgatattgggaaacgaatcaaggtcaaaaaactcagtcata  
atagttgaaatttaaaaattttatcaattcaatccgattgggttcgt  
tttggttatgggtcgggttctatatcatcaaaccaatcggtttgggtcct  
aaagataattataaatattaccaacaccagtggttaaacacatatca  
acaaacctaaagttagataaacaagaga.

146. An isolated promoter comprising the following nucleotide sequence:

aattggcactcttct tctgctgggttccaaaagaaacgaat  
caatatgtgcaacaagaagagctccagaagcagtcattctctaaaat  
cttaatctaacaacagctcaagaagaaaaaattccatagctagaga  
gaacacaaagtcacaagacgacgctcgtagaggcacaagtcaaacct  
gaatggcttaagccgaactgagtggttttgactagaccatcatcaga  
aaagctccaagacggtagtcggatgttagatcgctcaagtaatttt  
tggttttggttggtctcacgttctcagctgcccatttgatttcagttt  
gggcttttcttactctctaaaaggcccaatttcatttaggttagttt  
atttgatcattatccttactataaaggcttcgcctttcgagaaattt  
agggtttctctgtctgtctcgtcactcagggttggtgcctcaacgac  
tgcttcacttctagcttgattcttcttcttcgtttatatgtatactg  
tacattagattattcttggttctcgcagcttctgctatagattttgat  
tcttttttttggttggtctttgttctcgtttccaggatcagatcttagct  
aaattgagacaagctcaaaatgaggtacttgacgcacatctcttaoatt  
cactgtttaattagagaacaaataggtctctgaatcgtgattcagaga  
cgtattgttctctgtcatatgcaataagtttaattagagaacaata  
cgtctctgaatcgtgattgttctttggatgtgcgttattgatagctt  
tatgatgttaatagtctaggaatgacacgaagtgttctgcagtttt  
gcataaatgctctttactaaggcctctaaatttggtgacaaatcta  
aatcttgccctcataaaaaattt aggtgtattaagataagattattttg  
tatggttagtgctataatgtgggttggtcatgttgaggttgatcaatg  
ttgtgtatttttggttggttaagttaatttgcttaactctgttctttg  
tggttaatacagtaagcttcagagtgaggccggttcgtgaagccatc  
actactatcacagggaatccgaggcaaagaaacgtaactttgtcga  
gactattgagctccagatcggctcgaagaactatgacctcaaaagg  
acaagcgtttcagtggtatctgtcaagttaccacatatccccgctct  
aaaatgaagatctgcatgctcggagatgccagcatgttgaagaggt  
gatatacttttcatggaaattgatcattttgtgctctgtttcttgt  
ataatgggtttgtgctcatttcatttggtggctctatttagtttcatt  
tgatgttgatataatgtcttctgaatgtagatgcattgatgttttcggaa  
tttggtcattgtttatttaggcttcatttcttgacataattaaatatt  
tgcttatttcatcttgatcttctcgtaggctgagaagatgggggttg  
gaaacatggatgttgagtctctaaaaaagcttaacaagaacaagaa  
actcgtcaagaagcttgcaaaagaaataccatgctttcttgccctctg  
agtctgtcattaagcagattcctcgtcttcttggtcctggtcttaac  
aaggcaggcaagtcttggtcagcctaataattccattgttcttcttt  
acatccgttttgattttggatagggttttagtagtctatttcttttgt  
caatgtctttttgatacaatgccaatcctttatcctgtgagattatg  
cttctttgatgattcttaagt aacattcctttgctttactttacaca  
ggaaaatttcccaactcttggtgagccaccaggaatccttgaggtcaaa  
gggtgaatgaaacaaaggcaacagtgaggtccagctgaagaagggttc  
tgtgcatgggaggttgaggttggttaaccttt.

147. An isolated promoter comprising the following nucleotide sequence:

tggaactgagatataagaggaagggtgattttcatgcaa  
attttttttttttttttttttgaatgaatgcaaaatttattcaaaaa  
aaaaaacctgggtacatcaagtacttcatttctgagtttttgaaa  
aatctaaagacaacaaaagactttacaatttaataaaaaataataa  
aaatactttatcactctcaacgaaattggttgatttaataacgtatct  
cttggtaaaacagcggttttatttgacgaaattggtataaatgaataa  
aatgataatagaaactagtgtggtacgtaaaatacctctcatttggc  
aaaataacgggttatgtatcatgagatttgcatacgacagcggtgctta  
aatagtgtgctttcaggagaaaatatataccaagttatttgctgaaa  
ttaccacgcaaatctgaggttcgaatggcaaaaataaaaaaccaatgt  
catttccttaattgtattaaggtcatttaataaaaattgtacactttt  
ttcacctgtaagcggtccaaagtgtagaatggataactagaagggtc  
aaagggtataatattaataagcgaactcactttttgcccagtgattt  
cacttcttacatttgcttgataatagttacccaaaagtgtatatatat  
tcccttatacaattgttctattttctggattataaggggaataagaa  
aaaagaaaagagagagtataataataacttttataaagtgatgcta  
gatctctaatttgtaacgaaaagt tcaaagtgaagaaaaaacgaaaa  
agtttttctgtttttgttttatatctatagccaagaaagtttctcaga  
tttacaagaagttaactgagaaaacaaaaaaaacttatgaagca  
tgaaagactaattaacgaggtgatttaattttgagacaaattaaacat  
cgaattaaaagtaacatttggaggggtttatatgttatatatgtgaca  
tgataagtcagattcatgactaatgtatatctggaatctaactgga  
agaatagagaacgaagcagagccaaaggtcaacttgccagacacgaat  
caacagatttgtgaatgagaccaaatacaatgggtcataaacgggttggg  
tttaaacgggcaagtcaccttgggtcaattccattcggtatttcctt  
catgcaagacctctgatacaaccaaagactccattacaatatctt  
ttcgatcacgagctacttattttcaaatgtgttacctctttcgtgac  
tcttgtgttgtgtggtaaagcctagtcgagatgtgtcggtatatata  
ggcatatatatacaaatgcgacaaaataagttatatattgttttaa  
tttctatatattccatttctatatgcatggctgggatttttgacaaaa  
ccctaattcaagaatagaatcca aaagatgggatcaaagaatataat  
ctaattgggtgaccacattttccgatttaattcgcatagttaatatt  
ctttccactactttatgccgcagaaatttgtaattaagtaagacaaa  
gaaatacagatataagatgggtcgtagaaaccagtagaggaatttcat  
tttctgtggataagtggaaatttaataagagaatgggtctttactctt  
tacagtgggaaatgggaatagtagccattataatttcatcagattc  
tatatatgcatgtttgtataagctaaaataaatacgtttaagcattc  
ttcaaaaaaatttacaagttctagagactctcttaacgtcggcaatt  
tatattctactttacatgacactttcaggaaaagaaaactatactca  
ctagcagatcattaaattttctt tttctttttttgaatgaaccttag  
ttgtgggtttttatttttggtagctagaaacttcagtgtttttttcc  
gccaatggtagtgctttgatgatgggtccgg .

148. An isolated promoter comprising the following nucleotide sequence:

caatcaaggtaacgaaggaggatcagcgaaaggatgggcta  
tatttggagtttttctcgtgtcagtaatgctttgtgatcttcca  
tgcggacatataactgaagaataaactcaactcattgtgttctgggtg  
tggttcttctgatcagattcctcgttgcacatcgcacttttctgctgt  
gggggctttatttataaaacaagagtagagcgtgtggtaatcttcat  
atcttctacaaattccacttccaattctctaattattctctcacgtga  
tatacacacactcaatcactgatgtactcgtatggatgcagcgtgga  
actgatgcattgcccggggatgtcacttctatcgggcttactagaaac  
tgtaagtattacaagaaaactcaaaaggattccatttatgcaaaatc  
taagagaaaagctcactgtggtctttgggttacaatttatggatctctc  
aagagacaaatgctatgtaagctaatgattttgggtcttgataaaca  
ggtagagtggaaagtggacaaagctactcaagaactgaagacatcaaca  
atgcttttgccaatgaagtctcatgggaccgctcttccgcatcttct  
actcaagcgacaacaacacagagaccaagtgaagaacatatggtgc  
gatctaattttgtcaagtgcctcacaagaggtactgtttcaagccat  
ggtagtgacgcttgatctgcgatttctggattttgctttgtatg  
tttattttctacctcttagaaaagggtcaaaaagttaatagcttcac  
cgtgagaatgttgttttaccagattcatgtgctatgatagaaaaag  
acaaagcaaaacagagttcttcttcttggcttaggttacaagaacaaga  
gtatcgttataaaagtcaacaaagattgaaacatatattttgtcaagg  
agtgggtagaatctcttctactctcttgcctttctcactaagacaa  
aaaaaagacttggactttgtctaagggtttgtggatattattaacca  
agtccttttgcaaaaagtaatatgttttttcgcattcctcttttag  
aatttagtttaattctaggctttaatttggttattactttcttgaaaa  
atgatctgtttattctattcatacttgggttacctcgctttttatctt  
acttctacaaaaggattatcagtgaaagttagtctcttactctcacc  
ttccgaaaataaaacaaaaataatcgatacttctagatcaaaccaagt  
tgattaaaacatccctattccctacgattctgatcttgagatatatt  
atcatgttaagatctaaattgacaaagaaaactgatttttcatttcta  
gtaggaaaaataattactattagtgatcatgattgtcgaccgtaaga  
ggtaggtttagttactctccatcttctttgaagaagtcagaaagtca  
gaaattatatcaaattaaacatcaatattgaacacatatatctgtat  
ggttttatgtttagaaaattccaatatatttatatttcttagggaaaa  
agaagcttattcttcaaattattgttatgagtcgttaaaatatggat  
aaaaatataaagtctaaatatataaaaactcagtttgctttgctttta  
cctctccaagtcctccaaagtc aaattaatttttagttaattaaacca  
aaaagggttatttagtcaaacttagcatgcaatgctgggtaccaaacc  
caagcatttagtctcttttaactctctttttctccaataagtttttac  
aatttttaattgtttgcatttcccttgattatttatcttcatcccaa  
tttagctaataccaactccgttctcttattcttccaagtccttttcta  
taaatacgttctcttccctctcttattcatatcactcaccacaaag  
cttctctcatttctctcat .

149. An isolated promoter comprising the following nucleotide sequence:

atgttgtgagtgaaggagaagaagagggaaacaaaggtatt  
tatttgtagcgagttttgttttctgtgacgcggttttgtctgtgttcaa  
tggtgacgaaacgagtgagagagtgtctgattattaaagaaaaccct  
aattaagtcagacccgccggttataaaaaatagtcaaaaagtaggaaa  
acgcgtgtgtgagtgagacagagacagcccattgtttgctttatggg  
cttataagcgagacgtgttaaattgggctttttcctttatggccgaaa  
acaaaagaaacgtcgcctgagagattcgaactctcgcgggcagagcc  
catgtacttagcaggcacacgccttaaccactcggccaaagcgactt  
gttgctatgagttagacaaaaattcattaaaattctctattatgatttc  
tcatagtgtgtgtatattgtggatctactaaaaattctttgttat  
tattactttattttgtgaattagtttgatataggtaagtacaaagtt  
aactttattatttactcaaaaatttatcagattaactgattttatatt  
gtttccttttggtatatagacgtactatagtttttagaaaaaccataa  
gatttcctttatatattcatagagtgaagagatgagatgagatcttggc  
tggagaagaaataagtttccacgaggaggactcttttttttttggtga  
agacgaggaggaggactcttgggtgatccagtctttacgttagacat  
cgaccctacattttatgtgctttctctatcaacatggcaggtaaaa  
atcttcattcaaccgaaccaaataagtcctctcccaataatattca  
agcaccatcctttgggaaactcatacactacagtcacactcttt  
cattttctttcaacgctcaacttaacaaatgatatagtctagttgtc  
aattatatgttttaattagtgctttcacatcaaattctgggttgata  
tttgatgactattttcggaacatctcaatgtcccgcaaatacaatc  
gtctatcatatataatcccgtacgttgattctttatagatagaataa  
tatggcggtgatctttataataaacatatagaatcgtgtagatttat  
tttattttatttttatatatcgcataaattgcaaaatacttatatat  
gtttgttatatatgataccaattttatagttacttaaaaaaggttaa  
gcgataatatatatatcaactttttatacaaaaaaagtataaacac  
atggtaaaagaaaaataaaaaatgaagacatgggtgtgacacgaaaatgg  
cactaaatatacatatataatagatagctacaatatcccatcataca  
cacttttttaattgactaatacataacttacacacttttttaattga  
ctaattcataactttttatcaattgtcaacatgcaaatcatatttcc  
gttgaactattattcttattttgtttttaaaagaagggcttcctggg  
aataaaaaatagatttccaaaatgacgttagagcaaaaaaaaaaaaaag  
gttgtctgggtctggtaaaatgaaaaagcaaacgctcttggtatagaa  
aagtaatatactgectcctaaattctctcgtccttctaccgaagaatc  
tctccactcttgccctctttcgaaaccctaaaccagaagcaccagat  
tttttcaactttttcccagagaacaatagaaaacccaacttgtgtc  
tctaggggttttctttattccttctcatctttggattttcttggtca  
tcattttggaagcttaccacacagcgaaaaaattataacttccatcg  
attcctgggtctctctctctcgtctctctctgcatgtgctaaatcgccg  
gactgatcctcactgtcacctctgtt.



150. An isolated promoter comprising the following nucleotide sequence:

gattaggggtttgagttgtcactggaaagaggtttgattgt  
gagtgatgatggagagattatgaaggagtttgtgtgtatttatagag  
gagttaggggttttgaggtttgatgagaagtaggtttgaagaagtttt  
gttgttgcaacttatttagagttacttgttccacaaccacaagtaag  
attggtcacttctaagttctaactagaaacaacatgacacatggag  
atttcagctaacctagtttaa tgtatatgtattatattttatttaa  
tattataaaataaaataaattttcacaaataaaaagaactacaaaaa  
gtgagaaaaataatttgataa acaaatttagaaaaattagtatatcaa  
taaataaatttataatccgatgggttttgccttttggtttggcctttg  
tttgaacttcgatgagtgactatgtatagcgaaaacaattcgggttg  
tttttggtttaatttttaaaaaatacaagcgacaatatctgatgagaa  
taggtgaaaagcaaataatatcagtttaattggaaataattactttt  
ttacaactaataattttgttttggtcaaccaacaaatagatttaattaa  
ttatgggtttatgagcttttat tgtttgcgacagtatatatatgttaa  
aatagtgatattgcatggcggagaggtccggaagcaacacatatctcc  
tttttaatttttttttaacaagaataacatgttaatttttttttgga  
aattaataaagaatacatatttctaatttttgcgtcagatagatgat  
taaagagtggtgtgttttttttaacaaacaaggaatacatatatacata  
tttcatattttctctcgacattgtttgttttttaaaaaatagattaa  
agagttctacgaagctaagtagctaacgaagacttgaaatgagaagaa  
gacgagaatcttttaatatattttttgttaagcgataatatttgaaaa  
ttaataaatatagattaaaggaaataacaataacgcagatatcggtaa  
gtcatagaaaaaaagaaacaaacacaaacttacataaacatgtttcct  
aattttglaatggagtaaaattccttcttttttttttttttttgattt  
ggattccaattagtaaagaactcaatgactataaataacctttaacc  
ctctcattatttcttactatcaattgattaagctctcgttcctaaga  
aagcaatagacgaacaagaacccatcgaagaacacaaatctctcttt  
gaagttgtcgataatgttagtaacacgttacttcgtccaagactttt  
ttgccgttcggtttcttacaaacaaggatttggttaccattacttt  
tgtcgtaactcctttttacatgtacgtcaaaaagtggttcctcgctc  
cggcttgaagaaacgaccttc taccacacaaaagcttattttaaac  
cgtctaaaaccggaaaatctcaatctaaaccggatcgggttcatgag  
aaaccgattcaaacaccgagtggaagaagtagaattttttgatgggtc  
cgtcacaatgtgtgctgctcc tgcgcaagacatgtaccgattccga  
tattttgtggtgtaaagatga tcaaagagtcctcaaagctaagcacg  
acttgaatgagaagaagaaga ccaattactcaattagattttgtttt  
gtggagcaattattgtctatttatctttgttttttagcaaataatctg  
tatccactaatcttcacagtaacttgactaacaagaagtaaagagttt  
tcttattttccaattgttttttaatctgatacttttttcataatttta  
caatgtttgatgaaaaaaaacattcaaacctaaattttctttttttg  
gtatgaattcaaacctgaattacttttgacgaggaccgacgggtata  
aataggggtgatctcccaacaaaacaaaagggt.

151. A transgenic plant or transgenic plant tissue comprising an isolated promoter according to any of claims 143 through 150.

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APPENDIX 1

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
1, 2, 3	2293133	glyceraldehyde-3-phosphate-dehydrogenase
4, 5, 6, 7	7143495	Histone H4
8 & 9	7143515	ATP dependent RNA helicase, mRNA sequence
10, 11, 12, 13	7143527	nematode specific
14 & 15	7143602	protein serine-threonine phosphatase 1, catalytic subunit
16 & 17	7143612	40S ribosomal protein S4
18	7143666	cytochrome p450
19, 20, 21, 22	7143675	Neuroendocrine protein 7B2
23, 24, 25	7143839	nematode specific
26	7143863	40S ribosomal protein S17
27 & 28	7144016	vacuolar ATP synthase subunit G
29	7144025	malate dehydrogenase
30 & 31	7144060	J2 pcDNAII Globodera rostochiensis cDNA similar to Bystin, mRNA sequence
32 & 33	7144225	similar to arginine kinase
34	7144354	pyrroline-5-carboxylate reductase

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SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
35, 36, 37, 38	C10	ribosomal protein L18a
39, 40, 41, 42, 43	C118	ribosomal protein S11
44 & 45	C122	ribosomal protein L16/L10E
46 & 47	C127	FMRFamide-related neuropeptide precursor
48	C129	ADP-ribosylation factor 1
49	C130	ribosomal protein L11
50	C137	nematode specific; conserved in <i>C.elegans</i>
51 & 52	C138	ribosomal protein L7
53	C145	ADP/ATP translocase
54 & 55	C148	troponin
56 & 57	C154	calponin
58	C16	translation elongation factor EF1A
59 & 60	C18	40S ribosomal protein S16
61	C27	ubiquitin
62 & 63	C46	nematode specific
64, 65, 66	C48	ribosomal protein S3AE
67	C59	40S ribosomal protein S5/S7

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
68	C8	glyceraldehyde 3-phosphate dehydrogenase
69 & 70	C82	60S ribosomal protein L30/L7E
71	C90	glyceraldehyde 3-phosphate dehydrogenase
72	C135	nematode specific
73 & 74	C206	predicted troponin
75	C227	cytochrome P450
76	C238	vacuolar ATP synthase subunit G
77	C246	40S ribosomal protein S4
78	C308	FMRFamide-like neuropeptide precursor
79	C342	ubiquitin
80 & 81	C344	nematode specific; conserved in <i>C.elegans</i>
82, 83, 84, 85	C370	40S ribosomal protein S5/S7
86	C426	nematode specific
87	C458	histone H4
88 & 89	C481	ribosomal protein L30E
90 & 91	C556	nematode specific; conserved in <i>C.elegans</i>

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SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
92	C628	ribosomal protein S17E
93 & 94	C665	malate dehydrogenase
95 & 96	C669	malate dehydrogenase
97	C694	ribosomal protein S3AE
98 & 99	C709	ADP/ATP translocase
100 & 101	C714	ADP-ribosylation factor 1
102	C721	calponin
103 & 104	C726	ribosomal protein L11
105	C736	nematode specific
106 & 107	C773	troponin
108	C834	nematode specific
109	C860	bystin
110 & 111	C863	troponin
112 & 113	C883	translation elongation factor eEF-1A
116	C888	40S ribosomal protein S16
117	C898	glyceraldehyde 3-phosphate dehydrogenase
118 & 119	C935	peptidyl-glycine alpha-amidating monooxygenase
120 & 121	C937	calponin
122 & 123	C942	peptidyl-glycine alpha-amidating monooxygenase

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
124	C954	arginine kinase
125, 126, 127	C969	calponin
128 & 129	7235653	ribosomal protein L18A
130	8005381	neuroendocrine protein
131	7235496	pyrroline-5-carboxyla te reductase
132 & 133	7275710	protein phosphatase pp1-beta catalytic subunit
134	7923685	nematode specific
135	7641370	40S ribosomal protein S11
136 & 137	7923404	nematode specific
138	7797811	ATP-dependent RNA helicase
139	7143613	predicted phospholipase D

## Appendix 2:

### Exemplary genes used for RNAi vectors.

#### Promoters:

##### *Constitutive:*

##### **Super Ubiquitin from Pine**

CCCGGGAAACCCCT CACAATACATAAAAA AAATTCTT TATTTAATTATCAAACCTCTCCACT ACCTT  
 TCCACCAACCGTTA CAATCCTGAATG TTGGAAAAAACT AACTACATTGAT ATAAAAAACTA CATT  
 CTTCTTAATCATAT CAAAATTGTATAAATAATATCCACT CAAAGGAGTCTA GAAGATCCACTT GGACA  
 AATTGCCCATAGTTG GAAAGATGTTCA CCAAGTCAACAA GATTTATCAATG GAAAAATCCATC TACCA  
 AACTTACTTTCAAGAAAATCCAAGGAT TATAGAGTAAAA AATCTATGTATT ATTAAGTCAAAA AGAAA  
 ACCAAAGTGAACAAA TATTGATGTACA AGTTTGAGAGGA TAAGACATTGGA ATCGTCTAACCA GGAGG  
 CGGAGGAATTCCCTA GACAGTTAAAG TGGC CGGAATCC CGGTAAAAAGA TAAAAATTTTT TGTAG  
 AGGGAGTGCCTGAAT CATGTTTTTAT GATGGAAATAGA TTCAGCACCATC AAAAACATTTCAG GACAC  
 CTAATAATTTGAAGT TTAACAAAAATA ACTTGGATCTAC AAAAATCGTAT CGGATTTTCTCT AAATA  
 TAACTAGAAATTTTCA TAACTTTCAAG CAACTCCTCCCC TAACGTAAAAAC TTTCTTACTTC ACCGT  
 TAATTACATTCCTTA AGAGTAGATAAA GAATTAAGTAA ATAAAGTATTC ACAACCAACAA TTTAT  
 TTCTTTTATTTACTT AAAAAACAAA AGTTTATTTATT TACTTAAATGG CATAATGACATA TCGGA  
 GATCCCTCGAAGCAG AATCTTTTATCT CCTTGGTTTTGT ATTAAGAAAGTAA TTTATGTGGGG TCCAC  
 GCGGAGTTGGAATCC TACAGACGGCT TTACATACGCTT CGAGAAGCGTGA CGGATGTGCGAC CGGAT  
 GACCTGTATAACCC ACCGACACAGCC AGCGCACAGTAT ACACGTGTCATT TCTCTATTGGAA AATGT  
 CGTTGTTATCCCGC TGGTACGCAACC ACCGATGGTGAC AGGTGCTGTGT GTCGTGTCGGT AGCGG  
 GAGAAGGTCCTCATC CAACGCTATTAA ATACTCGCCTTC ACCGCTTACTT CTCATCTTTTCT CTTCG  
 GTTGTATATCAGTG CGATATTCTAG AGAGCTTTTCAT TCAACCCGG

##### **Strawberry Banding Vein Virus 1**

aagcttttctactgtgggttaatttcattaatctatccaggtgaaaacctcaaggaga  
 tctctcttctccaaaagacctctacagggcaatcaaaaactacagaaccagagttt  
 gtagtgcacagagtagaccaatctacctgagaatcacgagtagcttcctagagtggtg  
 aaaatgatgacatccttattccataccactggattgaggtaggactatccaatggaa  
 aaattccatgggacagagcatataagaagaccgcaacagtcgagtagcttcacagaga  
 taactgcactcagacctaaaaggataaaagcagtagatataatcagtagtactaagatct  
 tcgtagattcaagaagaagctt

##### **Strawberry Banding Vein Virus 2**

Gtttaaacacagcccaagataacagaaaaagtc aaagggtgttcgaaagaccacttgt  
 gactaaggatcatttcattccataat tatctggtagcacagactcatgataactgcga  
 ggaacacaagttctttacagtcgat tcaaagacactttctctttacggtttcattga  
 aggagccgacccagaatattgtcagagaagcttttctactgtgggttaatttcattaat  
 ctatccaggtgaaaacctcaaggagatctctcttctcccaaaagacctctacagggc  
 aatcaaaaaactacagaaccagagttt gttagtgacagagtagaccaatctacctgag  
 aatcacgagtagcttcctagagtggtgaaaatgatgacatccttattccataccactg  
 gattgaggtaggactatccaatggaaaaattccatgggacaagtcataaagaagac  
 cgcaacagtcgagtagcttcctcagagataactgcactcagacctaaaaggataaaagc  
 agtatataatcagtagtactaagatcttcgcagattcaaagaagaagcttaactatgc  
 ttagtacaagataattctaataagcaattattcagaattaatcaaggagaagaatt  
 aataactctttcagaatattgaagcccgctttacaagtggtcagtagctatcactga  
 aaagacagcaagacaatggtgtctcgatgcaccagaaccacatctttgcagcagatg  
 tgaagcagccagagtggtccacaagacgcactcagaaaaggcatcttctaccgacac  
 agaaaaagacaaccacagctcatcatccaacatgtagactgtcggttatgcgtcggt  
 gaagataagactgacccagggccagcactaaagaagaataatgcaagtggtcctag  
 ctccacttttagcttttaataattatgtttcattattattctctgttttgcctctat  
 ataaagagcttgattttcatttgaaggcagaggcgaaacacacacagaaacctccc  
 tgcttacaaccatgtattgtagctaaacctcttaggaggatattc



**Nematode Inducible:****Trypsin Inhibitor from Arabidopsis (clone#6598343)**

cccgggagcaagcaagaacaccagaGaagaagaaaagcactacagagaaaaatgtg  
 agcttaagecgtctccaacaacacttCtctgggagtctaaaggatgctgcaaaaagc  
 ctgggtggtagacttccgcatatttCcaagcatgggtttatttttggtagcacaca  
 aactatctgacctcgacttggatttCcttctgcagtttgtccaactacattgaaac  
 ggatatgcaggcaacatgggatcatgaagggtggccatctcgtaagattaacaaagtga  
 acaggtcactaaggaaaatacagacggtactggactcgggtccaaggtgtagaaggag  
 gactaaagttcgactcagcaactggcGaattcattgcagtttagacctttattcaag  
 aaattgataccaaaagggtctgtcgtCctcttgataatgatgcacatgcaagaagaa  
 gtgaggagatatgctgacgatactCattcaagctccaggaagctaaatctgtcg  
 acaatgccattaagttagaggaggatCcaaccatgaatcaagcaagaccaggtaaga  
 acttctctatccataaaccatagatggagcgattagaatcttaatccattttcagtt  
 tttgcaggatcattcatggagggttaaCgctagtgggtcagccatgggcttggatggcc  
 aaagagctctggcttgaatggcagtgaaGgaataaagagcgtttgcaacttaagctct  
 gtggaaatttcagatggaatggatccAcaatccgatgcagtgagcagttattgttgaa  
 cctaaaccaatccatgtcatgcagcatAtcagattcatcaaatgggtcagggcagtt  
 ctgctggaagctcatctacttccatGgaagattggaaccaaatagagaaccacaaac  
 agtaatagcagcgagagtggatcaacAacgctgatcgtaaaggccagttatagagaa  
 gacactgtacgtttcaagttcgagccAtcagttgggtgtcctcagctctacaaagaa  
 gttggaaaaagcttttaactgcaggaCgggtcgtttcagctgaagtacttggatgat  
 gaagaagaatgggtgatgctggttacAgattctgatctccaagaatggttggagata  
 ttacatgggtatgggaaaaacactcgggtGaagtttctcgttcgtgatttgtctgcccct  
 ctaggtagttctgggtggcagtaattggtTatcttggaacaggcttatgacgtcgttaag  
 acatagacacacacagttatgtattCCagtgaaagaatggttgtttatttctctaga  
 tattagttatgcttataaataggcatgaaggagaaagacaattttggtatagtggagt  
 tcagcagaaaaatgtatatgtttttcgtttttatatgaatcagagaataaaagtggga  
 tgttatatctacgttgctaattgttgtaCctgctcaccatctttcatataagaaaag  
 agaacacttttagttatccctgtgatGcagaatcgtattctttgttatctctccatt  
 cctgtggaaaccaacaaagtcaactaAatttcggtttaattgggttgggttttaagtc  
 aacgaggacttgatttttagttgggtCgggctataattgtgttcatttggtttt  
 tttccccccttatcagtttaacgtccaTatccatatctttttcttttttaacggcaa  
 agttcatatccatatcttatgatgtgCctaaaagagggagaagatgcgaagacagaa  
 ttttcatatttgaaagggttcgatatCgatattgggaaacgaatcaaggtcaaaaaa  
 ctcagttcaatagttgaaatttaaaaattttattaattcaatccgattgggttcgtt  
 ttgttatgggttcggttctatatcatCaaaccaatcggttgggtcctaaagataatta  
 taaatattcaccaacaccagtggttaaAcacatatcaacaaacctaaagttagataaa  
 caaagagaccggg

**Arabidopsis Transmembrane Protein from Arabidopsis**

(clone#6468048)

cccgggaattggcactcttcttctgcTgggttccaaaagaaacgaatcaatatgtgc  
 aacaagaagagctccagaagcagtcA tcttctaaaatcttaatctaacaacagctca  
 agaagaaaaaaattccatagctagagagaacacaaagtcaacagcagcgtcgtaga  
 ggcacaaagtcaaacctgaatggcttAagccgaactgagtggttttgactagaccat  
 catcagaaaaagtctccaagacggtagtCggatgttagatcgtcagtaatttttgg  
 ttttgggtctcacgttttcagctgCccatttgatttcagtttgggcttttctta  
 tctctaaaggcccaatttcatttaggtTtagtttatttgatcattatccttactata  
 aaggcttcgcctttcgagaaatttaggggtttcttctgtctgtctcgtcactcaggtt  
 tgtgcctcaacgactgcttcaactctAgcttgattcttcttctcgttttatatgtat  
 actgtacattagattattcttgtttCcgagcttctgctatagattttgattcttt  
 ttttgttgtctttgtttcgtttccaggatcagatcttagctaaattgagacaagctc  
 aaaaagaggtacttgacgcatctctAcattcactgtttaattagagaacaatcgt  
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61

ttatgatgttaatagtctaggattgacacgaagttgttctgcagttttgcataaatg  
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 atgttgagggtgtcaatgttgtgtatcttctgttctgtttagttaatttgcttaactct  
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 ccagatcgggtctgaagaactatgacccctcaaaaggacaagcgtttcagtggtatctgt  
 caagtaccacatatcccccgctctaaaatgaagatctgcatgctcggagatgccc  
 gcatgttgaagaggtgatatactcttctcatggaaattgatcattttgtgctctgttt  
 cttgtataatggttttgtgctcatctcatttgggtggctctattagtttcatttgatg  
 ttgtatatgtcttctgaatgtagatgcatgatgttttcggaatttggctattgttta  
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 gtaggctgagaagatgggggttggaaaacatggatgttgagtctctaaaaaagcttaa  
 caagaacaagaaactcgtcaagaagccttgcagaagaataccatgctttcttggcctc  
 tgaatctgtcatttaagcagattcctcgtcttcttggctcctggctcttaacaaggcagg  
 caagtctctggctacagctaatactcattgttcttctttacatccgttttgattttg  
 gataggttttagtagtctatttcttctgtcaatgtcttttgatacaatgccaatcc  
 tttatcctgtgagattatgcttcttctgatgattcttaagtaacattcctttgcttta  
 ctttacacaggaaaattcccaactcttgtgagccaccaggaatccttggagtcaaag  
 gtgaatgaaacaaaggcaacagtggaagttccagctgaagaaggttctgtgcatggga  
 gttgcagtttgtaacctttccggg

**Diaminopimelate Decarboxylase from Arabidopsis  
 (clone#4159709)**

cccgggtggcacaactgagatataagaggggaaggtgattttcatgcaaatTTTTTTT  
 tatttttttttgatgaatgcaaaatttattcaaaaaaaaaaacctgggtacatc  
 aagtacttcttctgagttttgaaaaatctaaagacaacaaaagactttacaatt  
 taataaaaaataataaaaaacttctatcactctcaacgaaattgttgatttaataa  
 cgtatctcttggtaaaacagcgttttatttgacgaaattgttataaatgaataaaat  
 gataatagaaaactagtgtgtacgtaaaaatacctctcatttggcaaaaataacggtta  
 tgtatcatgagtatgcatacgacagcgtgcttaaatagtgtgctttcaggagaaaa  
 tataaccaagtattttgctgaaattaccacgcaaatctgaggttcgaatggcaaaa  
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 ttttttcacctgtaagcgttccaaagtgtagaatggataactagaagggtaaaggt  
 ataataattaataagcgaactcacttttggcccaagtgtatttcttactttacatttgc  
 ttgatatagttaccctaaagtgtatataattcccttatacaattgttctattttct  
 ggattataaggggaataagaaaaagaaaaagagagagtataataataacttttata  
 aagtgtatgttagatttctaatttgtaaacgaaaagttcaaagtgaagaaaaaacgaaa  
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 agttaactgagaaaaacaaaaaaaacttatgaagcatgaaagactaattaacgag  
 gtgattaaattttgagacaaattaaacatcgaattaaaagtaacatttggagggttta  
 tatgttatatatgtgacatgataagtccgattcatgactaatgtatatctggaatct  
 aacatggaagaatagagaacgaagcagagccaaggtcaacttgccagacacgaatca  
 acagattgtgaatgagaccaaatcaatggtcataaaaccggttgggtttaaacgggca  
 agtcatccttgggtcaattccattcgttattccttcatgcaagaccctctgatacaa  
 ccaaagactcccatataaatattcttctgatcacgagctacttattttcaaatgtgt  
 tacctcttctcgtgactcttgtgttgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgt  
 atataggcatatatacaaatgcgacaaaataagtataattatattgtttaatttct  
 atattccatttctatatgcatggctgggatttttgacccaaaaccctaattcaagaat  
 agaatccaaaagatgggatcaaagaatataatctaattgggtgaccacattttccga  
 ttttaattcgcatagttaataattcttccactactttatgccgcagaaatttgaatt  
 aagtaagacaaagaaatacagataaagatgggtcgtagaaaccagtagaggaatttc  
 atttttctgtggataagtggaaatatataaagagaatgggtcttactctttacagtgg  
 gaaatgggaatagtagccattataatttcatcagattctatatatgcatgtttgta  
 taagctaaaaataaacgtttaagcatttctcaaaaaaatttacaagttctagagac  
 tctcttaacgtcggcaatttatattctactttacatgacactttcaggaaaagaaaa  
 ctatactcactagcagatcattaaattttctttttcttttttttgatgaaccttagt  
 tgtggtttttattttttagctagaaacttcagtgtttttttccgccaatggtag

tgctttgatgatggtccggcccggy

**Peroxidase from Arabidopsis (clone#4006885)**

cccgggcaatcaaggtaacgaaggaggatcagcgaaaggatgggctatatttggagt  
tttttctgctgtaagtaagtctttgtgatcttccatgcggacatataactgaaga  
ataaactcaactcattgtgttctggtgtgtttcttctgatcagattcctcgttgcat  
ctgcacttttctgctgtgggggctttatttataaaaacaagagtagagcgtgtggtaa  
tcttcatactcttctacaattccacttccattctctaattattctctcacgtgatata  
acacacactcaatcactgatgtactcgtatggatgcagcgtggaactgatgcattgc  
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aaaggattccatttatgcaaaatcta agagaaagctcactgtggtctttggttacaa  
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aacaggtgagtggaagtggacaaagc tactcaagaactgaagacatcaacaatgctt  
ttgccaatgaagtctcatgggaccgc tcttccgcactcttctactcaagcgacaacaa  
cacagagaccaagtgaagaaacatatgggtgcgatctaattttgtcaagtgcctcaca  
agaggtactgtttcaagccatgggatggcacgcttgtgatctgcgatttctggattt  
tgctttgtatgtttattttctacctt ctagaagagggtcaaaaagttaatagcttca  
ccgtgagaatgttgttttaccagat tcatgtgctatgatagaaaaagacaaagcaa  
acaagagttctttctttgcttaggtt acaagaacaagagtatcgttataaagtcaac  
aaagattgaacatattttgtcaaggagggtggttagaatctcttctactctcttg  
ccttctcactaagacaaaaaaaga cttggactttgtctaaggttttgtggatatt  
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tttagtttaattctaggctttatattgggttattactttcttgaaaaatgatctgttta  
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tgaagttagtctcttactctcacct tccgaaaataaaacaaaaatatcgatacttc  
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aaaaataattactattagtgatcatgattgtcgaccgtaagagggtggttagttact  
ctccatctttctttgaagaagt caga aagt cagaaattatatcaaattaaacatcaa  
tattgaacacatatatctgtatggtt ttatgtttagaaaaattccaatatttatatat  
tcttagggaaaaaagaagcttattctt caaattattgttatgagtcgttaaaatatgg  
ataaaaaataaagtcataatattaa aaactcagtttgcttttgcttttacctctcca  
agtctccaaagtcaaattaatttttagtttaattaaacaaaaaagggtttattagtc  
acttagcatgcaatgctgggtaccaa acccaagcattagtccttttaattctcttt  
ttctccaataagtttttacaattttt aattgtttgcatttcccttgattatttatct  
tcattccaatttagctaataccaact ccgtttcttattcttccaagtcctttcctat  
aatacgttcttcttccctcttat tcatatcactcaccacaaagtccttctattt  
cctcatcccggy

**Mitochondrial Uncoupler from Arabidopsis**

(clone#4220510)

cccgggatgttgtgagtgaaggagaa gaagagggaaacaaaggattttattttagc  
gagttttgttttgtgacgcggtttt gtctgtgttcaatgttgacgaaacgagtgaga  
gagtgctgattattaaagaaaacc taaattaagt cagaccgcggttataaaaaat  
agtcaaaaagtaggaaaacgcgtgtgtgagtgcagacagagacagcccattgtttgct  
ttatgggcttataagcgagacgtgt t aattgggcttttctcttattggccgaaaaaca  
aaagaaacgtcgctgagagattcga actctcgcgggcagagcccattgacttagca  
ggcacacgccttaaccactcggccaa aagcgacttgttgctatgagttagacaaaatc  
attaaaaattctctattatgatttct catagtgtgtgtgtatattgtggatctactaa  
aaattctttgttattattactttat tttgtgaattagtttgatataggtaagtacaa  
agttaaactttattatttactcaaaat ttatcagattaactgattttatattgtttcc  
tttggtatatagacgtactatagttt ttagaaaaaccataagattcctttatatttc  
atagagtgaagagatgagatgagat cttggctggagaagaaataagtttccacgagg  
aggactcttttttttgggtgaagacgaggaggaggactcttggttgatccagtcctt  
acgttagacatcgacccctacattt atttgctttctctatcaacatggcaggtaaa  
aatcttcatccaaccgaaccaacca aagtccttcccaataatattcaagcaccatc  
ctttgggaaactcatacatactacagtcctacactctttcattttctttcaacgctca  
acttaacaaatgatatagtcagttgt caattatatgttttaattagtgttttcaca

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tcaaatctcgttggatatttgatga ctattttcggaaacatctcaatgtcccgc  
 atacaatcgtctatcatatataatcc cgtacgttgtattcttatagatagaataata  
 tggcgtgatctttataatataacata t agaatcgtgtagattttattttatttt  
 ttatatcgcataaattgcaaaata ctatatatgtttgttatatatgatacccat  
 ttatatgttacttaaaaaagttaag cgataatatatatatatcaactttttataac  
 aaaaaagtataacacatggtaagaa aaataaaaaatgaagacatgggtgtgacacgaa  
 aatggcactaaatatacatatataat agatagctacaatatcccatcatacacactt  
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 ttatcattgtcaacatgcaaatcat atttccgttgaactattattcttattttgtt  
 tttaaaagaagggttctcgtgtaata aaaaatatgatttccaaatgacgttagagcaa  
 aaaaaaaaagggttctcgttctggtt aaatatgaaaaagcaaaagcgtcttggtag  
 aaaagtaataactgcctcctaattt ctctcgtccttctaccgaagaatctctccact  
 ctctgccctctttcgaaaccctaaacc agaagcaccagattttttcaactttttccca  
 gagaacaatagaaaacccaacttctgct ctctctagggtttttctttattccttctcatc  
 tttggattttcttgggtcatcattttt ggaagcttaccaccagcgaaaaaattataa  
 ctctccatcgattcctggttctctct ctctcgtctctctgcatgtgctaaatcgccgg  
 actgatcctcactgtcacctctgttccggg

**Stress protein from Arabidopsis (clone#6598614)**

cccggtgattaggggtttaggtgtg t cactggaaagagggtttaggtgtgagtgatgat  
 ggagagattatgaaggagtttgtgtgt t atttatagaggagtttaggggtttaggggtt  
 gatgagaagttaggttgaagaagttt t gttgttgcaacttatttagagttactgtt  
 ccacaaccacaagtaagattgggtcac t tctaagttctaactagaaacaacatgaca  
 catggagatttcagctaacctagttt aatgtatatgtattatattttattttaaatat  
 tataaaataaaataaattttcacaaa taaaagaactacaaaaaagttagaaaaataa  
 tttgataaacaaatttagaaaaattag t atatacaataaataaatttataatccgatgg  
 ttttgccttttggcttttggctttgtt t gaacttcgatgagtgactatgtatagcgaa  
 aacaattcgggttgttttgggttaa t tttaaaaatacaagcgacaatatctgatg  
 agaataggtgaaaagcaataatatc agtttaattggaaatatttacttttttaciaa  
 ctaatattttgtttgggtcaaccaaca aatagatttaatttaattatgggtttagagct  
 tttatttgttgcgacagtatatatat gttaaaatagtgtatttgcattggcggaagggt  
 ccggaagcaacacatatctccttttt aattttttttttaacaagaataaacatgttaa  
 ttttttttgaattaataaagaata catatttctaatttttgcgtcagatagatga  
 ttaagagtggtgtgttttttttaaca aacaaggaatacattatacatatttcatatt  
 tctctcgacattgtttgtttttttaa aaaaatagattaaagagctctacgaagctaagt  
 agctaacgaagacttgaaatgagaaga agacgagaatcttttaatatttttgttaa  
 gcgataaatattttgaaaattaataaa t atagattaaaggaaataacaataacgcagat  
 atcggtaagtcatagaaaaaaagaaa caacacaaacttacataaacatgtttcctaa  
 tttgtaatggagtaaaattccttctt ttttttttttttggatttggattccaatta  
 gtaagaactcaatgactataaataa cctttaaccctctcattatttcttactatca  
 attgattaagctctcgttcctaagaa agcaatagacgaacaagaacccatcgaagaa  
 cacaaatctctcttgaagttgtcga taatgttagtacaccgttacttctgtccaaga  
 cttttttgcggttccgttcttaca aacaaggatttgggtaccattacttttgcg  
 taactcctttttacatgtacgtcaaa aagtgggttctcgtcctcggttgaagaacg  
 accttcttaccacaaaaagcttat t taaaccgtctaaaaccggaaaatctcaatc  
 taaaccggatacgggttcatgagaaac cgattcaaacaccgagtgagaagtagaatt  
 ttttgatgggtccgtcacaaatgtgtg ctgctccttcgccaagacatgtaccgattcc  
 gatatttgtgggtgtaagatgatcaa agagctctcaaagctaagcacgacttgaat  
 gagaagaagaagaccaattactcaat tagattttgttttggaggcaattattgtct  
 atttatctttgttttttagcaataat ctgtatccactaatcttcacagtacttgact  
 aacaagaagtaagagttttcttat t tccaattgttttttaactctgatactttttc  
 ataattttacaatgtttgatgaaaaa aaacattcaaaccctaaattttcttttttgg  
 tatgaattcaaaccctgaattactttt gacgaggaccgcggtataaatagggtgat  
 ctcccaacaacaaaaagggtcccggtg

**Pectinacetylesterase from Arabidopsis**

(clone#6671954)

cccggtggtggggacaatggatccggtctgctgtagcaacaaggctgaaaaagatta

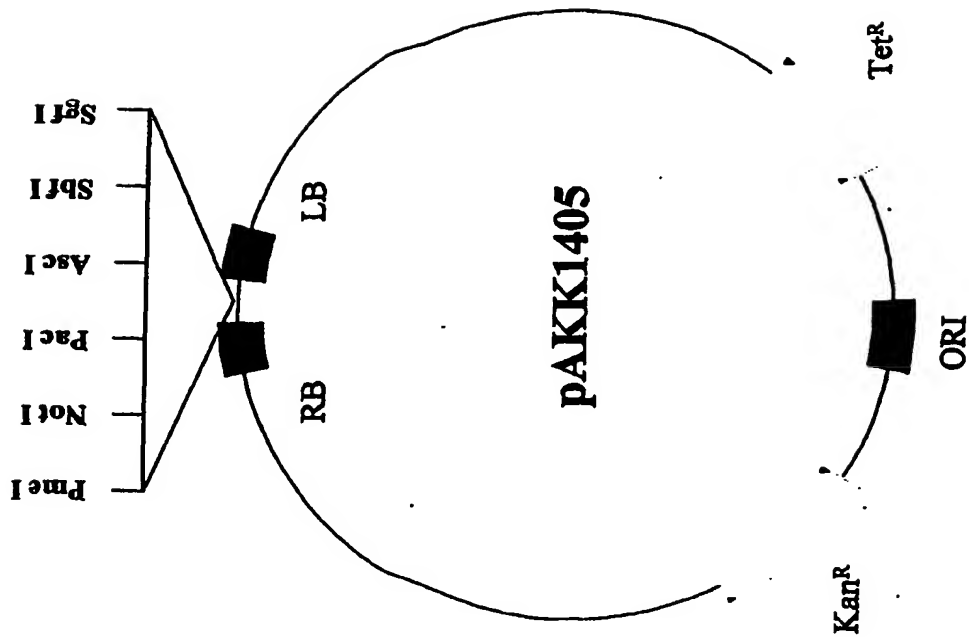


FIG. 1

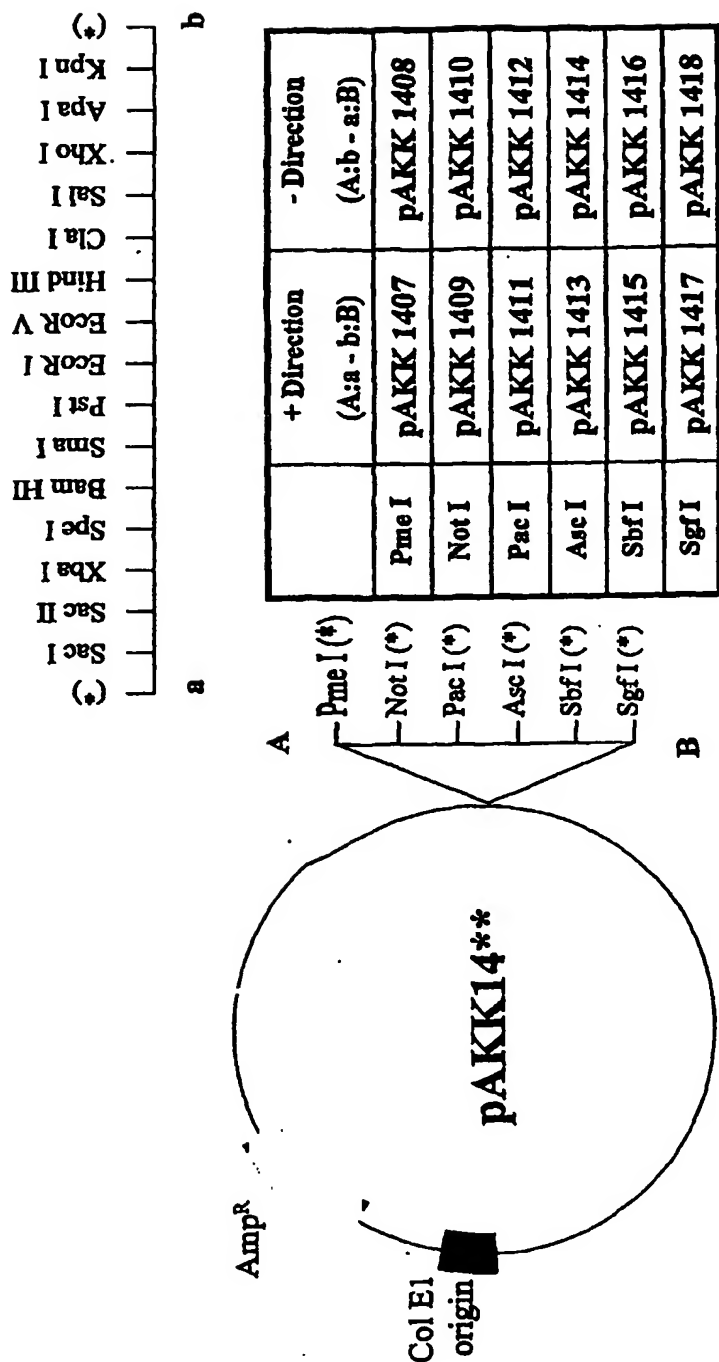


FIG. 2

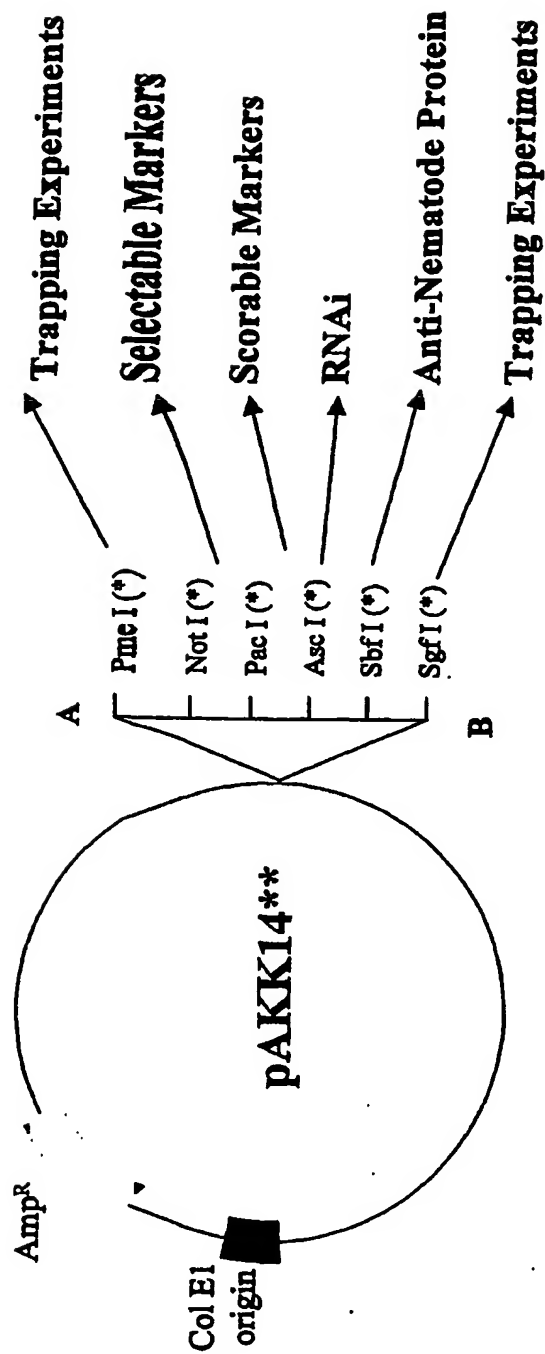


FIG. 3

# Selectable Markers

pNOS / NPT-II / tNOS

pSU / Bar / tNOS

pSU/ Intron / Bar / tNOS

pUBQ3 / Intron / PMI / tNOS

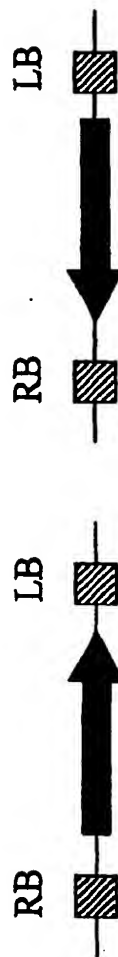
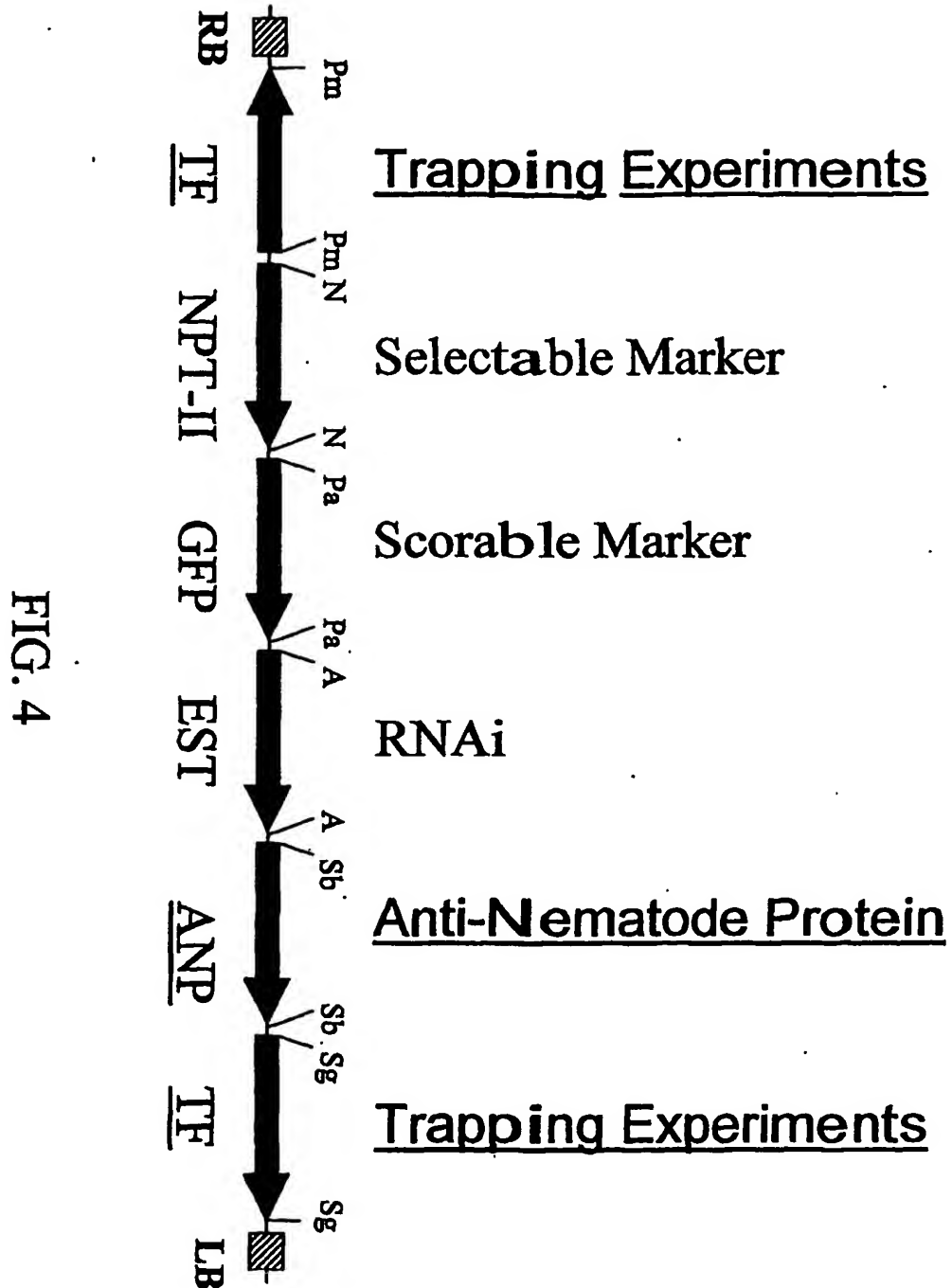


FIG. 5





## Scorable Markers

Base Construct	Markers
/ Intron / Marker / tNOS <sup>1</sup>	GFP
pSU / Intron / Marker / tNOS <sup>2</sup>	GUS
pSBV1 / Intron / Marker / tNOS	NLS-GUS
pSBV2 / Intron / Marker / tNOS	PAP1C

<sup>1</sup> Construct useful for promoter analysis.

<sup>2</sup> Construct useful for high constitutive expression of genes of interest.

FIG. 6

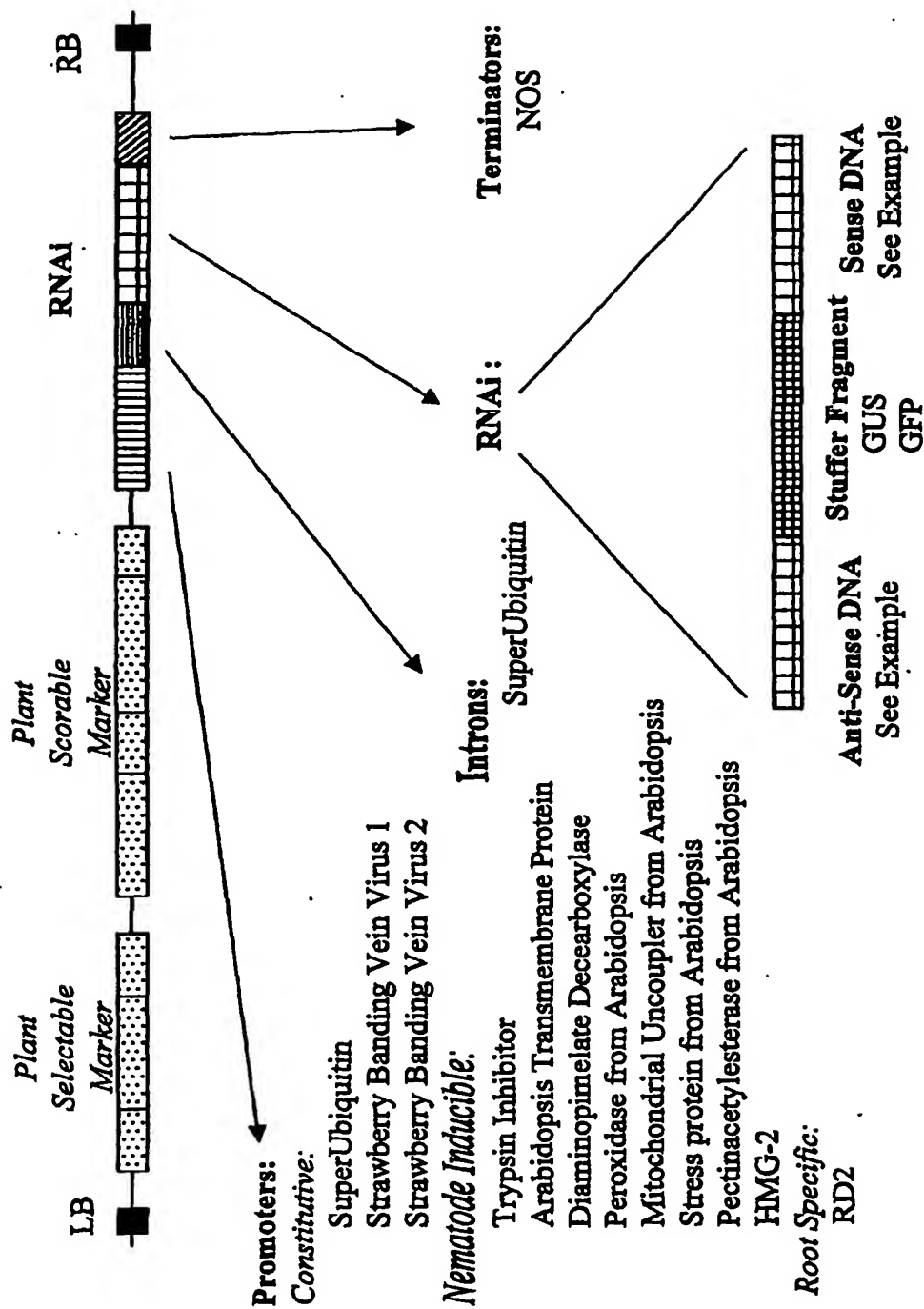


FIG. 7

AKK110P1  
SEQUENCE LISTING

<110> Mushegian, Arcady R.  
Taylor, Christopher G.  
Feitelson, Gerald S.  
Eroshkin, Alexey M.

<120> Materials and Methods for RNAi Control of Nematodes

<130> AKK-110P

<140>  
<141>

<160> 139

<170> PatentIn Ver. 2.1

<210> 1  
<211> 165  
<212> DNA  
<213> Globodera rostochiensis

<400> 1  
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taaacaatagc aaaaatgggt aaaccgaagg tcggcattaa tggctttgga cgcattgggc 120  
gcttggcggt gcgcgctgcg gttgagaagg acaccgttca ggtgg 165

<210> 2  
<211> 342  
<212> DNA  
<213> Globodera rostochiensis

<400> 2  
cgactacatg gtatacatgt tcaactacga ctcgacccat ggccgcttca atggcaaaat 60  
ttcgacaagc gccggcaatt tggctgttga gaaagagggg aaggccacgc acaccatcaa 120  
ggtgttcaac ctcaaggacc cggccgagat caaatgggct gaggtgggcg cggaatatgt 180  
gatcgagtcc accgggggtgt tcaactacat tgagaaggct tcggcacact tgaagggggg 240  
cgccaagaag gtggtcatct ctgctccgtc cgctgatgca ccgatgtacg tgatgggct 300  
caacgaggac aaatatgacc cggccaaggga caacgtgatt ag 342

<210> 3  
<211> 205  
<212> DNA  
<213> Globodera rostochiensis

<400> 3  
gaagccggcc tcattggacg ccatcaaggc ggcggtgaag aaggctgccg aagggaattt 60  
gaagggcatt ttgggttaca cagaggacaa ggtggtgtcc acggactttc ttggagacag 120  
tcgctcgtcg atcttcgacg ctggggcgct catctcgttg aaccgcact ttgtcaagtt 180  
ggtcagctgg tacgacaatg aattt 205

<210> 4  
<211> 167  
<212> DNA  
<213> Globodera rostochiensis

<400> 4  
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tcgtccattt gtcaattgtg gccctaaa ga gggccgtttg ggtagtttt ttggtgttcc 120  
ttctccttgc tggctcaacc accgaagcgc tacagcgtcc ggccttg 167

<210> 5

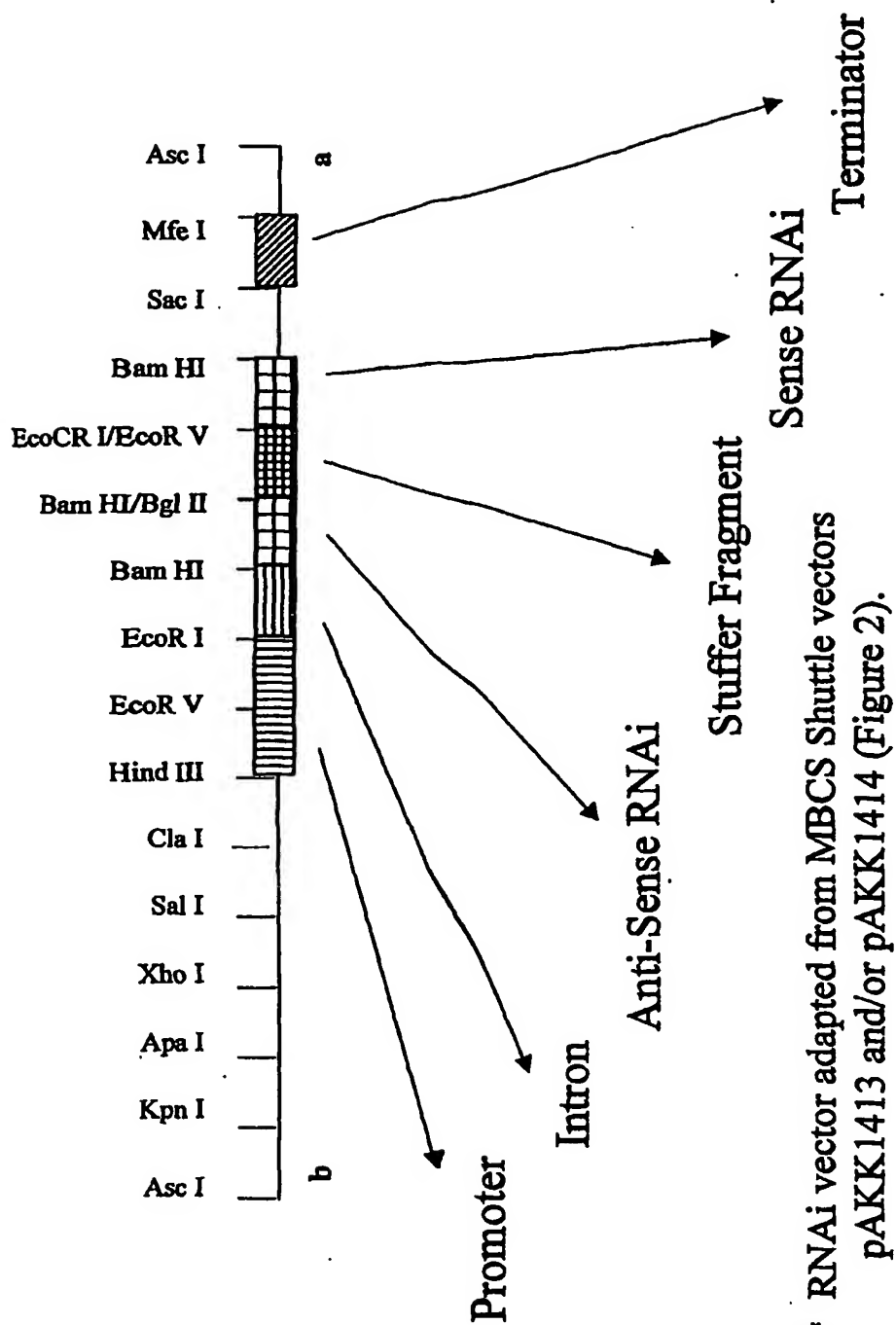


FIG. 8

## AKK110P1

<211> 41  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 5  
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<210> 6  
 <211> 79  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 6  
 gtttcccagg aaaactttca gcacggaaCg agtctcctcg taaatgaggc cagagatgcg 60  
 cttaacgcct ccacgacgg 79

<210> 7  
 <211> 168  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 7  
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 ctttccgagt ccttttccgc cttttccgCg tccggacatt ttgttgtaa atcagaagag 120  
 cacagagagt aggagaaata ggaaattttg cctcgtgcg aacgtgcc 168

<210> 8  
 <211> 330  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 8  
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 atacgagcga ttcaccaagt acatgccggg agtgaagggt tccgtattct tcggagggat 120  
 gccgataaag aaagacgaag aggtattgCg taagaacacg ccgcacattg tcgtcggaac 180  
 gccgggacgt cttttggcct taggacgcac tggacatctg aagctgaaag gcgtcaaatc 240  
 ctttgtgctg gacgaatgcg acaaaatgat tggagatgcc gacatgcgcc acgacgtgca 300  
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<210> 9  
 <211> 136  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 9  
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 aaggaaaatg agaaga 136

<210> 10  
 <211> 141  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 10  
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 ttgtgttca tcactttctt cagcagcgac aatacggcca atccggtgaa agggccaaag 120  
 tcaatagctc gctcgggtacc t 141

<210> 11  
 <211> 141  
 <212> DNA

## AKK110P1

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 17

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ttgagaagac aaacgaaacg ttctgtctgg tgtacgatgt gaagggccgt ttgtcatcc 180
atcgaattca aaagctggag ggccagtaca agctgtgcaa agtgaagaag caggccgtcg 240
gggacaagca ggtcccctac attgtcaca c atgacgcgcg caccattcgc taccggaccg 306
ctcatc

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&lt;210&gt; 18

&lt;211&gt; 528

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 18

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gaattcgcac aacgaattga agacttatgc ggcagaaaaa ggacttttgc caaagtgtga 60
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gttcctaccg tatacaaacg ctgtaataa tgaaacaatt cgattagtca atttgatccc 180
gttcaatctt agccatttgg cgcttgaaag tatgcaaat ggcaatttta ttgtgaagcg 240
tgggacacca attgtaccgc aggtcagca tgttctgttc gacgaaaaac tgtatccgga 300
gcccgatcgg tttttgcccg aacgctttc ggacgatgag ggccgtttga agaaaagcga 360
cgaacttatt gcatttgggg ttgggaaaaa gcaatgtgcc ggcgaaagctt tggcccgaat 420
gacacttttt ctgtttgccc ctaatttct tctcgccctac aaagtctctc cgtccgatcc 480
actgaatcct ccaagcctga aaaagtggc ggattatctg ttacaca 528

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&lt;210&gt; 19

&lt;211&gt; 335

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 19

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gaattctttg agaaagcggg aattcgtttc tggctataaa atgattctgt gggccacgat 60
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ggcgccgatg ttccccgact cccagttca cgatttgatt tcgcgcgaca tcgaatcctt 180
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acagctaggc cccgaggggc cttttgagca gcggcaacag gtgaagagtg acaatgttct 300
ccccgcgtat tgcgagcctc caaatccctg tccga 335

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&lt;210&gt; 20

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 20

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ggacggctgc acggaacagt tcgagaaca tgccgagttt tcgcgcagct ac 52

```

&lt;210&gt; 21

&lt;211&gt; 190

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 21

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gcttgtgtga ccaggagcac atgtttaact gtccgtcgaa gaacaaccgc gaggagtacg 60
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aattccatct cagcgggcg gaggagccg gccgtcgaaa acgctcttgt cgcccggctt 180
cggccaaccg

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&lt;210&gt; 22

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

<400> 22  
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<210> 23  
<211> 54  
<212> DNA  
<213> Globodera rostochiensis

<400> 23  
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<210> 24  
<211> 77  
<212> DNA  
<213> Globodera rostochiensis

<400> 24  
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aacagaccgg aacagca 77

<210> 25  
<211> 439  
<212> DNA  
<213> Globodera rostochiensis

<400> 25  
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acaacgtgca gcagcaacat gttgttggtc aacaacagca gcaacaacag aatttccaac 180  
aaccgcccgc cctatcgtag actcacagcc accaacaaca aaaacaacca ccacaagcgt 240  
cacagtcgat gttgtcaatg aaaagtggca atgtgtgcgt tgtgttccg caacaatcgc 300  
agcagcacca ctaccaacag cggacactga cgccactgaa gcacacatcc gcatcctcca 360  
cgtccgatcg cttcgtcatc accaaaacca acaggggtgct tccactcccg tcgcagcaag 420  
gcgccacggc cactgatga 439

<210> 26  
<211> 539  
<212> DNA  
<213> Globodera rostochiensis

<400> 26  
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cctcgacttt cacaccaaca agcgcatttg cgaggaggtg gccattatcc caagcaaacg 180  
gatgcggaac cgaattgcgg gatttatcac acatctgatg aagcgcattg agctgggccc 240  
tgtccgtggc atttccatca aattgcaggga ggaggagcgc gagcgtcgcg acaattacat 300  
gccgaaatc tcttacctgg atgcgcagaa tcaccagatg atcagcaccg accaagagac 360  
gaaggatatg gcggaatttc tggggctagg cctcaacttg gaagtgaag ggcctttgac 420  
gagtggcggc gctggcgtag gacgtcgttg agtcaggaca attggcata ttgttgaaaa 480  
atcatcgatg tttgttcgc atttggatga taatgcgctg ataaattttt gttgatttt 539

<210> 27  
<211> 179  
<212> DNA  
<213> Globodera rostochiensis

<400> 27  
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cggccgaaaa gcgtgcggca gaaaagatta atgatgccg gaagcgaaaa gcacagcgac 120  
ttaagcaggc caaacaagaa gccaggcgg agatcgagca gtatcgnag gagaggag 179



## AKK110P1

<210> 28  
 <211> 133  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 28  
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 gctgcagttg gtg 133

<210> 29  
 <211> 482  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 29  
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 caaaaggcga tgtgtttggc aaagatcag caattgttct cgttctctc gacattccac 180  
 cgatggccga agtactctct ggtgtccatt ttgaattgat ggactgtgcg ttggcaaac 240  
 ttgccggtgt ggaggctgtg accacggaa agcaggcctt caaggacatt gactacgctt 300  
 ttcttgtcgg agcgatgcc cgaagagag gaatggaaac aaaggacctt ttggcggcaa 360  
 atgtcaaaat tttcaagtcc caaggcgaag cattggccc cttttccaag cccgtncgtc 420  
 aaagtctctg tgggtgggcaa cccggccaa acgaacgcgt acatttgcg aaaatatgac 482  
 gg 482

<210> 30  
 <211> 605  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 30  
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 ttttctgcca ccggcacacc gtcacaatgc caaagggtca acactttggt gctgttgcca 180  
 cgactccgtg atgagattga cgagtacaa agactaaact ttcatttcta tcagtgttgg 240  
 tttaaagcaa tgttcaagcc ggccggattt ttttaaggga ttattttgcc tctttgcaaa 300  
 tctggcactt gcactctccg tgaagccat atctttgggt ctgctctgcg aaagatttca 360  
 ataccgcaac tccacgccc gtcagcaatg ctacgcatag caaaaatgga ctactcgggc 420  
 gccatttctt ttatcctacg tgttcttgtt gaaaaaaatt acacacttcc tttccgagca 480  
 ttagacggcc tcgtttttca ttttcttggg atgcgctcac atcagggcga gctgccagt 540  
 atttggcacc agacactgtt ggcttttgt gagcgttacg caaaagacat aagtgcagaa 600  
 cagag 605

<210> 31  
 <211> 112  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 31  
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 aatgaggaaa gtgaagcaaa tgtgcccgtt tatgcgcgta atgatgaaat gg 112

<210> 32  
 <211> 105  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 32  
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 ttgacaaaat cgaggcgggt tacaagaagc ttcaggaagc gtctn 105

<210> 33

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<211> 425  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 33  
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 tacgctcctg acgctgaggc ttacacctt g ttcaagccgt tggtagaccc gatcatcaac 180  
 gactaccatg gtggcttggg tccgggcag c aagcagccgg caactgacct tggtagcggc 240  
 aaaacgcana tgctgaccgg atctcgacc c cgaggggaaa atttatcaat ttcgacacgc 300  
 gttcgttgcg gccgtttcct ttaagggata cccggttcaa cccgtgcttg acnaaaggan 360  
 aactacnttt ggagatggga aacnaaggc c nagggccgtt ttctaacatt ttnaagggcn 420  
 atcct 425

<210> 34  
 <211> 581  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 34  
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 tttgacggtc attccaagca gccataaac c caccaaaacc aaatacccc ccccaatcga 180  
 tccccccct ccaattctc cgcattatc c gcattatcaa ttctaatacag cacaaccact 240  
 gcatcattcc ttcccgcacc atacgatgc t aagtgaact ttgaaaattg gcttcatcgg 300  
 agccggaaaag atggccaag cattggcaag aggacttatc aattcggggc gataccggc 360  
 agagaatttg atggcgagtt gtccaaaga c ggacgaggct ttactggagc aatgcaaaaa 420  
 attgggaatc ggaacgacgc acgacaaca c tttggtcgcg cgagagaacg acgtcatcgt 480  
 attggcggtc aagccgatgc acatcagcaa agtgacgtcg gaaatcgac ccaatttccg 540  
 gagggaaacat ttgcttattt cattgattag gaattacact t 581

<210> 35  
 <211> 102  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 35  
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<210> 36  
 <211> 34  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 36  
 tgcaaatgat gcaaacccca cgcttcacaa gatg 34

<210> 37  
 <211> 100  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 37  
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 aacacacgga gagatcgtt cgtgtcaaga ggttttcgag 100

<210> 38  
 <211> 176  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 38

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 gctcgtcacc gcgctcaggc ggatcgaaTt caaatcatca aagtgcaaac ctcaag 176

<210> 39  
 <211> 155  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 39  
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 agcgcgcgtt ccaaaaacaa ccgatcgtTt ttctgaacga caagttcaga acgcaaggga 120  
 ttgggaagaa ggcattccaac aaggaccgtTt actgg 155

<210> 40  
 <211> 35  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 40  
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<210> 41  
 <211> 70  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 41  
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 gcggacgatt 70

<210> 42  
 <211> 85  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 42  
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 cgtgttccg agatgtctct ctccg 85

<210> 43  
 <211> 193  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 43  
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 attctgagtc ggccaagcca accgcgaaCg gtcatttgtt atgggttcta attgttgctg 120  
 tttttcaatt attgtgtta aatgactgaa tttatgatca acggtatact agtattcttc 180  
 tgaaaaagct cga 193

<210> 44  
 <211> 219  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 44  
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 gaagacgtcc ggcgcgttgt tatcgtata ttaagaacaa gccgtatccg aagtcgcgct 120  
 ttgtcgcgg tgtaccgac ccaaaaattC gcatttttga ttggggtaga aagcgcgcca 180  
 ccgttgacga attcccatgc tgcgtgcata tgatatcga 219

## AKK110P1

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 <211> 489  
 <212> DNA  
 <213> Globodera rostochiensis

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 atgttgctct ggcgtggtgc ggaccgtctc cagactggga tgcgtggtgc gttcggaaaag 180  
 cctcagggac tcgtggcgcg tctcagcatc ggtgatatgc tgatgtcagt gcgtattcgt 240  
 gaccaacacc aagctcacgc attggaggcg ttccgtcggg cttaaattcaa gttccctggt 300  
 cgtcaataca tcgtcttctc ccgcaagtgc ggcttcacca aattcgatcg cgaggatatac 360  
 gagaaatacc gcaaggaggg ccgtgttatc cctgacgggtg tgcattgcaa gttactcaag 420  
 caacacggac ccgctgaagg agtggctcaa gaacccatt taatcttctg tttgtcttgt 480  
 gactcttgg 489

<210> 46  
 <211> 101  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 46  
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<210> 47  
 <211> 485  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 47  
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 acccgccgct ttattttgtg ttccagaaa acttgccgtt ggagcggccc ttcgacgagc 180  
 aaaaacgagg ctcaggaggag gaattagccg aagaagcgat gggaacgaag gcgaagaggg 240  
 cgcaaacggt cgtccgattc ggcaaaaggg cgcaaacatt tgtgcggttc ggaaagcgtg 300  
 cacaacatt tgtacgcctc ggaagggaca cgcaaggga attcgatggg aaaatgcaaa 360  
 gtgaacagca acagaaaaag gcttaaagca aacggcgggc acttttctt taatgaatgc 420  
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 taaca 485

<210> 48  
 <211> 651  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 48  
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 ctgctggaaa gacgaccatt ctgtacaagt taaagctcgg cgaaattgtc accaccatcc 120  
 caacaattgg cttcaacgtg gaaaccgtcg aatacagaaa catctcgttc actgtttggg 180  
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 gactgatctt cgtcgtggac agcaacgatc gcgagcgtgt gggcgaggcg cgtgaagagt 300  
 tgatgcgaat gctggcggag gacgagttgc gcgacgcggt gttgctggtg ttcgctaaca 360  
 aacaggattt gccgaatgag atgaacgccg ccgaactgac agacagactt ggactgcaca 420  
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 ctgttgcaat tgcccgcgga attgatgacg attgaattta tttgtgtgtt tgcgcgcgca 600  
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<210> 49  
 <211> 660  
 <212> DNA  
 <213> Globodera rostochiensis

## AKK110P1

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tgcacttgca ccaaaagtgt gcccacttgg attgtcgccc aaaaaaattg gtgaagacat 180
tgcaagggcc acacaggact ggaaagggct taagggtacc tgcaagctga caattcagaa 240
tcgtgtcgcc aagatcgacg ttgtcccatc ggccgcctct ctgatcatca aagagttgcg 300
cgaacctccg cgagaccgca aaaaagtcaa aaacgtgaag cacaatggca acctgacctc 360
cgagcaagtg atcaacattg cgcgtcagat gcgccctcgt tcaatcgcac ggaagttgca 420
gggcaccgtg aaggaaattt tgggaaccgc ccagtcgggt ggctgcacca tcgatggaca 480
acatccgcac gacattgtgg acgcgatcag agggggagac atcgaaatc ccgagggaata 540
aagaaaggac ggcgcctccg attttgtgg gacggacatt ggaatttga ggtgaatgag 600
ttgccaaatt cattcattca tcaattgttg ttattgntgg tacggataaa ttgttaattg 660

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&lt;210&gt; 50

&lt;211&gt; 625

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 50

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gtgccggaac agacgctcga ggaggttagc cgtctgcagc ggacgagctc cttgttggac 60
gtggcaatcc gggacggcgt cccctaccct ccactgcctc ctacaaaccg atcccccgaa 120
tacatgaaca tgcctgaccg ctcttctctc gtgccaaatt tccgcatcta ctcgggcgcc 180
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aaagcgaacc cgcactattg gcactaccgc cacacctttt gggactatcc ctaccagggc 420
aatggttctg actacgacaa cctcccaat taccggccct actacaacca tcgccttaac 480
ggatatgctc ggccgtatca ctaccggtcc catgcgctgg cccaccggt caattaccg 540
gaaggaatgg tcaggaaacg ggtctgacaa atcgaaactgc tccaaattga cgtgtccgc 600
attcgaaaga agacgaaaaa agctt 625

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&lt;210&gt; 51

&lt;211&gt; 402

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 51

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gaattccaag tttagcaac attttgaaaa tgaccgaagc caaaaaactt cccgaggtgc 60
cggaactttt gctcaagcga cgcaaaatca gagctgcgca aaaggccgca aaagcaaaaga 120
acaaattgag ttctatcaaa aaagcacgga ccaagaaggt ggaaatcttc aaaagagccg 180
agcagtattt ggtggagtac cgtcagaagc aacgccaatt gcttgcgctg aaacgtgaat 240
cgaagaaagt cggcaattat tatgtgccag aagagcccaa actcgctttt gtgggtccgaa 300
tcaaaggcat caataagatt catccgcgtc ctgcgaaggt tctgcagctt ctccgcttgc 360
gtcagatcaa caacggcggt ttcgtaaagt tgaacaaggc ga 402

```

&lt;210&gt; 52

&lt;211&gt; 433

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 52

```

ccgaccgta catcgcttgg ggttatccga gtcagaagat catccgtcag ttggtctaca 60
aacgcggtta cgccaaagag aagggacagc gcattccaat aacggataac aacattgttg 120
agcgcagttt gggcaagcat gacgtgattt gtgtggagga tatgatccat cagatttggaa 180
ccggtcggac cgcacttcaa acaggtgacc aacttcctat ggcctttcaa gctgagcaac 240
ccggtgggcg ggttcaagaa gaagtccaat cacttttgtg gagggaggcg attatggaaa 300
ccgcgaggac caaatcaaca aattattgga aagaatggtc taatggaagg gaagcggana 360
aagaaaggaa attgnggcgt ttttctgttg ttgttttgac gataaattgt taactccaaa 420
aaaaaaaaa aaa 433

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&lt;210&gt; 53

&lt;211&gt; 768

&lt;212&gt; DNA

AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 53

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tccggtggta	ctgcggcggc	tgtctccaaa	actgttgttg	ctcccatga	acgtgtcaaa	180
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ctgggacttt	gcccgtagcg	gtttggcccg	tcgatgtccg	aaaagctggg	tcccgcgagt	540
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accgcggctt	cttcgtctcc	gtccagggca	tcatcattta	ccgcgccgcc	tactttggat	660
gctttgacac	cgcgaaagatg	attttcgcgc	cggatggcaa	gcagatgaat	ttcttccica	720
catgggcat	cgctcaggtc	gtcaccgtgt	cgtccggtgt	cctctcct		768

&lt;210&gt; 54

&lt;211&gt; 338

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 54

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ttacattatg	gccacccaaa	ttggacaaa	tatgaacgcg	atggagcagg	actttgacga	180
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cgacgagttc	tgcgcgttgg	tgtacacggt	ggccaacact	gtggacaagg	acactctgcg	300
aaaggagctg	aaggaggcat	tccgactctt	tgacaagg			338

&lt;210&gt; 55

&lt;211&gt; 267

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 55

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gacggcagcg	ggaagatcga	attcgaggag	ttctgggagt	tgatggcggg	cgaaaccgac	120
tgagaaaaga	gcaaattcgat	ccaaatccaa	acggaccctg	cccatttcac	ctccatccgt	180
ccgtcgtatt	attatatatt	ccagtggaa	tttccatta	aaattcgggt	aaagtaaaat	240
aatttgacga	aaaaaaaaa	aaaaaaa				267

&lt;210&gt; 56

&lt;211&gt; 597

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 56

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catcccctac	cagatgggtt	cgaacaagta	cgcctcgag	aagggtcatga	ccggttttgg	120
acagccccgt	tgggaggtgc	ttgaccgctc	catctcgtag	cagaaccgca	agtcgcaagg	180
aatggttcgt	ctacagtcgg	gtaccaaccg	gttcgcctcc	caggcgggca	tgaccggctt	240
cggcacaccc	aggaacacca	cctatgaggc	ggaggcaggc	gagctgccct	acgaggacat	300
gaagaagtcg	gaggcgatca	tcccgtccca	ggccggttgg	aacaagggcg	actcgcagaa	360
gttgatgacc	aacttcggca	cgccccgtaa	caccaccacc	aaggtcaaag	tggagaattt	420
ggcggaattt	ccggaggaca	ttttgctgaa	aggacacggc	gaggtgcgcc	tgacgtccgg	480
taccaaccgg	ttcgcgtccc	agaagggtt	cgtcgcgttc	ggtaccggac	gtgacgtgtg	540
ccgtgagggg	gtgaacgtga	acgtgctgc	gggcgacttg	gagccgcttc	cggagga	597

&lt;210&gt; 57

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

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 ttcggtacgg gcccgctgtg 80

<210> 58  
 <211> 513  
 <212> DNA  
 <213> Globodera rostochiensis

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 ttgtcgaact gattccgacc aagccgatgt gtgtggaggc attcactgac tacgcaccgc 180  
 tcggccgttt tgctgttcgc gacatgaggc anactgttg cgtgggcgag atcaaatcag 240  
 tggagaagac ggaaggcggg ggcaagtga ccaagccagc gcagaagggtc ggcgcgactg 300  
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 aaaatgcgac caacctcttg tttatcgttg tcttattcag ttccttcac ccgtctctat 420  
 ccatattgtc gttagcttg ataattgtt attttttgtt attgtcctgg ttggaaaata 480  
 aatttggtca attaaaaaa aactcggtcc gaa 513

<210> 59  
 <211> 393  
 <212> DNA  
 <213> Globodera rostochiensis

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 aaaaggggca agggcttgat caaggtcaat gggcgctcct tggactacat gcagccggag 180  
 attctgcgca ttaagctcca ggagccaat ctcatgttg ggaaggacaa atttgaggga 240  
 atcgacatac gaatccgct caagggcggg ggacacattg cgcaaattta tgcaattcgc 300  
 caagcactgg ccaaggcact ggtcgcttc taccagaaga atgtcgacga gcagagcaaa 360  
 aaggaaactga aggagcaatt tgttgctta c gac 393

<210> 60  
 <211> 154  
 <212> DNA  
 <213> Globodera rostochiensis

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 taagaaataa tttttagat caaatgttt gatgatgatc cttgtttttg ttgttgataa 120  
 aaaaaattta taaaaaaaaa ccgccgata c tgac 154

<210> 61  
 <211> 666  
 <212> DNA  
 <213> Globodera rostochiensis

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 cgaggctagc gataccatc agaactgaa agccaagatc caggacaagg agggcattcc 180  
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 gcgtctgac ttcgccggaa aacagctcga agacgggcgc actctggccg actacaacat 480  
 ccagaaggag tccactctcc atctcgtct gcgtcttctg ggaggagaga actgaatcgc 540  
 gggctgatgg aaagatgac aatatgatg ctattcgatg acttgtctct ttcgatataa 600  
 ttgattgtg tccatttgtc ggtcatcaaa tctttatgac cccctcattg ggcattggaac 666  
 gataaa 666

## AKK110P1

<210> 62  
 <211> 213  
 <212> DNA  
 <213> *Globodera rostochiensis*

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 gtttgaggag acacattcgt tcgcaagaat ggctcgaaga taccgggcag aatttggtat 180  
 ggaaccaccg cagttggacc aagtgaagaa gtt 213

<210> 63  
 <211> 488  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 63  
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 cagctgagaa tggccagaat gtgagccgga ggacctgaag atttatgaac gaaattttcc 180  
 agtgaagtgg accaacgctc ttcgacttta tctgctttgt gtaaagtgtg tagaatcggc 240  
 ttccaattca aaggcttttc attccccaac ttttattttt gcgcaaaaaa tttcttagga 300  
 taagcgtgaa taattttattg atttgttttt tctttctttt atctccgcct cgaagtcgca 360  
 agtgttcctt ttggcccgtt cccttttgtt ttgaatgtta ttccattccc atcccctcac 420  
 tttctcatat ttgtgacatt cagctgcatt gttcgcactcc catttaaaag ttgagtgaag 480  
 tgcgattg 488

<210> 64  
 <211> 249  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 64  
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 gkgdyrbwnt msnwrmanrg artsstsgaa ttcccaagtt tgagagtaaa tattatttagc 120  
 taataatggc agtcggaaag aataagagaa tgggcaaaaa gggagccaag aagaaggctg 180  
 tcgatccgtt cacacgcaa gaatggtaCg acatcaaaag gccggcgatg ttacacatc 240  
 gaaatssts 249

<210> 65  
 <211> 362  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 65  
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 ntmsnwrman rgartsstsg tcaaccgtac tcagggaacg cgcatttcga gcgactttct 120  
 aaaaggccgc gtttacgaag tgtactggg tgaccttaac agcactgacg ccgactttctg 180  
 aaagtccgc ctgatctgtg aagaggtaca gggcaagatt tgcctgacca actttcacgg 240  
 aatgtcgttc actcgggaca aactgtgctc tattgtcaag aagtggcaca cgctcattga 300  
 ggcgaatgtg gcagtgaaga ctaccgacgg tttcatgctc cgactctttt gtatcggts 360  
 ts 362

<210> 66  
 <211> 128  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 66  
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 atgggtggaga tcatgcagaa agaggtctct tccggcgatc ttgaangaaa gtagtcaaca 120  
 agcctgat 128



## AKK110P1

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 <211> 502  
 <212> DNA  
 <213> Globodera rostochiensis

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 ttggtcagcc ttgacgttac cgagggtcaaa ctgttcggaa aatgggtccct taacgatgtg 180  
 gaagtgtccg acatttcgct tgtggattat attgctggga aggaaaaggc ggccaaatat 240  
 ctgcccgcaca gcgccggccg ttaccaacag aagcgtctcc gcaaggccac ctgtccggtg 300  
 gtggaacggt tgtctttgtc aatgatgatg cagggcgga acaacggaaa gaaactaatg 360  
 gcggtgcgca ttgtgaaaca ccccttcgag atcatcacct gctaccggag agaaccagc 420  
 ccaagtgttg gtcaatgctg tgataaacag tgggcccnc gaagattncac cactatcgg 480  
 acgtgcgggc actgttcgtc ga 502

<210> 68  
 <211> 519  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 68  
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 ttaatcatta aaactacatt taaaatatac tttttagaga atgtcgtcta aaatattctt 120  
 ttctcccctt tatgcatcta tctaaccaga cttggaagca atatggctaa tcaagtcaac 180  
 aatacggcag gaatacccaa actcgttatc ataccagcta accaatttaa caaatgcgg 240  
 gttgagaacc ataagagcct cggcgtcgaa aatagacgaa tgagtgtcgc caagaaagtc 300  
 ggtagaaaca acctggtcct cagtatatcc agaataccct ttaagctttc cttccgaagc 360  
 agtcttaatt gcattcttaa tagcctcctt cgttgctggc ttctccaaac gagcagtc 420  
 atcaacaacg aaaacgtttg ggcgtcggca cagaaaaagc catttcgggt aagcttccca 480  
 tccaattcat ggattgacct ttccaacagc ctttgcagc 519

<210> 69  
 <211> 218  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 69  
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 ttggagaata ttaagaaggc taagggttaa acgcaagtta tctttaaacg tgctgagcaa 180  
 tacttgattg catatcgacg taagcaaaa caagagtt 218

<210> 70  
 <211> 293  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 70  
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 taagggaaatc aacaaggta atttaaattt gctataaagt ttaggatggg tttagacaat 120  
 tcttctcttt taatgctttc taactttttc aaaaaagtt tgattttatc acccattaat 180  
 ctacaaattc ttttaatttat cagatccatc ctgctctctg aaaagttctt caacttttcc 240  
 gcttgcgtca aatcaacaat ggagttttca ttaaattgaa taaagctaca atc 293

<210> 71  
 <211> 422  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 71  
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 caggttgttt ctaccgactt tcttggcgac actcattcgt ctattttcga cgccgaggcg 120  
 taagttttga ttttctaaga ttataattaa ctttttaaat ttttcagtct tatgggtctc 180

## AKK110P1

aacccgcatt ttgttaaatt ggtagctgg tatgataacg agtttgggta ttcctgccgt 240  
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 gaaaagaata ttttagacga cattctctaa aaagtatatt ttaaatgtag ttttaatgat 360  
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 tg 422

<210> 72  
 <211> 374  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 72  
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 tgagccagtt aaactggact tcaacgttcc gcttattagt gattgggctg ctgcttctga 120  
 gtggcctcaa gaagaggaag ctgaggttgc acctactgca ccaattggtc agccacagcc 180  
 tcaacagcag caaactcaac aaggaggtga ttggaactct ggtactagt gatggtgaag 240  
 ggcaggaaaa ttgatagaaa gagaaatta t tatggaataa atgtaataa tgttgtgtgc 300  
 tgatttattt gttacatata caacaagttc ttttttgttg tttatttaat aaaagtttgt 360  
 aattaaaaaa aaaa 374

<210> 73  
 <211> 120  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 73  
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 agctttcaaa ttttgttttt tgattactct ttaacaaga ttcaactgat ggatctactg 120

<210> 74  
 <211> 369  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 74  
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 cagacccgct ccttttaact gctgtatct a aagaaaaccc tctaggcaac gtcccagttc 300  
 cactcaaatt caattttgtt aaatttttg cagatctaag tccttcttcc ttttgaacga 360  
 attgaactg 369

<210> 75  
 <211> 529  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 75  
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 aatctaaata aggtcttatt ctaagttta t attttcttt tacataaacc gtcaaccctc 120  
 caagtttttc aatgcttggg ggttttaatt g gatcctctgg taataatttg taggctagaa 180  
 aaaagtgttc agcaaaaagg aaaagcatc a ttcttgctaa ggcttctcca gcacattgcc 240  
 tttcccccac accaaaagct attagctcgt cagctttttt taatttccct tcattgtcta 300  
 tataacgttc agggtaaaaa ttttgggga t ttgggtatat ctttggatca aaaagaacat 360  
 ccgatacttg gggatcata aatgtacct t taggcaacac aaactttcca acatcaaat 420  
 cttccaaggc taaatgcccc aaattgaaa g ggactaaatt aacgagtctt aatgtttcat 480  
 taacaacagc atttgtataa attaattta g gtctgtgttc caaactaat 529

<210> 76  
 <211> 449  
 <212> DNA  
 <213> Meloidogyne incognita

## AKK110P1

&lt;400&gt; 76

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gaaaggcaca acgacttaaa caagcaaaa aggaagcgca agctgaaatt gacaaatata 180
gagaggaaacg tgaaaaacgt tttaaagagt ttgaacataa ttacctcggc gctagagatg 240
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ttgctgctaa taaacagcag gtaattgtt gtctacttca acttgtctgt gacattcgtc 360
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tagttgattg attataaaaa tgaaattga 449

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&lt;210&gt; 77

&lt;211&gt; 643

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 77

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taatcgctca aaatatgcac aatcttataa tgaagctagg atgatttgca aacaacgtct 240
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gggacgtgtt ggtattggtt gacatcgtga acgccaccct ggt 643

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&lt;210&gt; 78

&lt;211&gt; 584

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 78

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acaaacattt gttcggtttg gaaaaaggg acagactttt gttcgatttg ggagagattc 480
aaaacatcaa cataacttgt cagatcagaa gcagttaaaa actgacaaac aataaaaatg 540
atgaattatt taaaaatttt tttaatgat ttttaattaa aatt 584

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&lt;210&gt; 79

&lt;211&gt; 556

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 79

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aggttgaggc ttctgatacc attgagaatg ttaaggcaaa aattcaagat aaagagggta 120
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tggttgatta taacatccaa aaggagtcta cacttcaact agttttacgt cttcgtgggtg 240
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gtcgttcac caatgttgct acttctgggg cgggacgccg tcgtggccct aactccaacg 420
ctgcataaga gaatggctgt atcttgatga atgtatgggt atataatcaa ttaatacat 480
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&lt;210&gt; 80

AKK110P1

accatctcc

429

<210> 86  
 <211> 435  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 86  
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 actggagtag gtgtacttag tgttttagaa aaggcaaaat tagtttggtg gtttgaagag 240  
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<210> 87  
 <211> 501  
 <212> DNA  
 <213> Meloidogyne incognita

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 aattgttctt tagccacca atccgtaaag agtacgtcct tggcgtttca acgcatagac 240  
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 aatagctttc actccaccac gacgtgccaa tcgcccggatt gccggtttgg tgataccttg 420  
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 <213> Meloidogyne incognita

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 agaactagtc tcgagttttt tttttttttt tttttaanaa ttaacaattt atctcatttt 180  
 cctcttccat gaaaattaac aaaaagacga caacttaatc ccataattaa catcattttt 240  
 aagcttcagt cggcatgctt cgaataatgt 270

<210> 89  
 <211> 286  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 89  
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 agtttagcct ttccagaacg aagagctctt aacgtctgct ttagagccaa acaatacttg 180  
 cccgatttgg taacctggc gagacgagca ttgatatttt ctgtggactt tttctgtttt 240  
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<210> 90  
 <211> 391  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 90

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 <212> DNA  
 <213> Meloidogyne incognita

<400> 80  
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 tttccgtat agaaattatc gaggtacac actgacggat gcttactggt acgaccgtta 360  
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 ctac 424

<210> 81  
 <211> 89  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 81  
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 caacanatta ccgccattc ttgaccca 89

<210> 82  
 <211> 168  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 82  
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<210> 83  
 <211> 67  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 83  
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 ccagtac 67

<210> 84  
 <211> 42  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 84  
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<210> 85  
 <211> 429  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 85  
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 catcaactt ttaccattgt tacgtccatg catcatcatc gaacaaacca aacgttcaac 180  
 aatcggacaa tgagcctttc gaaaacgtt gatttgatat cgaccagcac tgtgcggaac 240  
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 gatatcgctt aaagaccatt taccaaacaa ttttaattca ggaaaatcaa ttgtagtcac 360  
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## AKK110P1

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&lt;210&gt; 91

&lt;211&gt; 131

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 91

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attacacatc a 131

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&lt;210&gt; 92

&lt;211&gt; 571

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 92

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tgctttcttg acagttttga gagaaccgat t 571

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&lt;210&gt; 93

&lt;211&gt; 671

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 93

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aggtggcacg g 671

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&lt;210&gt; 94

&lt;211&gt; 289

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 94

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289

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 <213> Meloidogyne incognita

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 aaagggagat gttttcggga aagaaacgcc catcgttctg gtaatgttgg atattcctcc 180  
 aatggccgaa gtgcttaag gagtggaact tgaaactttac gattgtgcct tggcaaatct 240  
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 <211> 323  
 <212> DNA  
 <213> Meloidogyne incognita

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 tttgtgcata atatgcagca gaaaaaattc cgacaaagaa tttcagcgct atgactcgtc 240  
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 <211> 717  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 97  
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 <213> Meloidogyne incognita

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 aaaaagaagg atggcttcga tgccaaaaag tttgcgattg atttggcttc tggagggaact 180  
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tcaaaatcga aacagattgt tttaaacggt tgaaattt

758

&lt;210&gt; 99

&lt;211&gt; 154

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 99

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tataatcaaa	ctgttcctca	aagttatgcc	catt			154

&lt;210&gt; 100

&lt;211&gt; 125

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 100

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tcgta						125

&lt;210&gt; 101

&lt;211&gt; 219

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 101

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&lt;210&gt; 102

&lt;211&gt; 473

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 102

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&lt;210&gt; 103

&lt;211&gt; 114

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 103

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&lt;210&gt; 104

&lt;211&gt; 255

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita



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 <212> DNA  
 <213> Meloidogyne incognita

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 agatagtgat ggacttgata tggctaaaaa tattttaaat tgaataaagg aaaaagaagc 180  
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<210> 107  
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 <212> DNA  
 <213> Meloidogyne incognita

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<210> 108  
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 <212> DNA  
 <213> Meloidogyne incognita

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caa

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<210> 109  
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 <212> DNA  
 <213> Meloidogyne incognita

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<210> 110  
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 <212> DNA  
 <213> Meloidogyne incognita

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aaattgagga gtatcaacgt ttcttcgata tgtttgaccg tggaaagaat ggctatatta 180
tggccactca aattggggta attatgaatg ctatggaaca agattttgat gaaaaaactc 240
ttcgaaaatt aatccgaaaa ttcgacgcag acggcagcgg caaatcgaa ttcgacgaat 300
tctgcgcctt ggtatacact gtggcgaata ctgtagataa ggacactttg cggaaagaat 360
tgagagaagc ttttcgtctc ttcgacaagg agggtaatgg ttacatctct cgtccaacac 420
tcaaaggatt actccacgaa atcgccccag acctcagcga taaagacttg gatgcc 476

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<210> 111  
 <211> 189  
 <212> DNA  
 <213> Meloidogyne incognita

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<400> 111
cgaagacgga agcggaaaaa ttgaatttga agaatttttg gaattaatgg ctggagagac 60
tgattgaaat ttaatttaga gatgaataaa aaattaacta aaatattttg ccataaaatt 120
ttggaagtgc ccaaaaattg cttttttgag aatttttatt ttaacgtct aaataatgaa 180
taaattggat

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<210> 112  
 <211> 164  
 <212> DNA  
 <213> Meloidogyne incognita

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<400> 112
ttgaggaaat ttaatttttt aaacaaatat aataattacc aaacaacaaa aaagaatccc 60
aaaaacaaca tttttaaatc aaatgacaga catatatttg caataacgat gtgtggattt 120
tctttttttt taaataatta acatcttaag cctgctattt cttc

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## AKK110P1

<210> 113  
 <211> 539  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 113  
 cagctttctg cgcagatttg gtaacctttc caccagcttc gaccttctcg acggccttga 60  
 taacaccaac agccacagtt tgacgcatgt cacgaacggc gaagcgtcca agaggagcgt 120  
 agtcagtaaa agcctcaaca cacattggct tgggtggaat taagtcgaca ataccagcat 180  
 ctccagtcctt caaagccttt ggattgtctt caaccttctt tccagttcga cggtcgacct 240  
 tctctttaag ctacgcaaac ttgcaagcaa tgtgagcagt gtgacagtca agaacaggcg 300  
 tgtagccagc agcaatctgc ccaggatggt tcatgatgat aacctgagca gtgaattgct 360  
 tggctctctt tgctgggtca ttcataagat cagaagtgcac tgaaccacgt cggatgtcct 420  
 tgacagagat gtcttaacg ttaaattcaa cattgtcttc aggaacagct tcagggagag 480  
 actcgtggtg catctcaaca gatttaactt cagtagaaat tccttcagga gcaaaggta 539

<210> 114  
 <211> 314  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 114  
 gtttttaatt ttagaaaatg tctacagaaa cagaaaagga tttagaacgt tgggaggatg 60  
 tccgtcgatt tactgagatt ggttcttcta aatttgccca tcccgtttt gttccaagcc 120  
 cggagaaatct tgaaagagta aggaaatgtc cagttttggt tgttggtgct ggtgngcttg 180  
 gatgtgaaat tttgaaaaat ttggccttat caggatttca aaatattgaa gttattgata 240  
 tggacacaat tgacctttca aatctcaaca gacagttttt gtttcgtgaa cacgatgttg 300  
 gcttatacaa agca 314

<210> 115  
 <211> 200  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 115  
 ttcgaagacg tggttaaagga tgtcgtctta ctgcacataa ttgtaaaata caagataaag 60  
 gacttgactt ttatgggcaa ttttcaatta taatttgtgg actagattct attgatgtc 120  
 gaagatgggt aaacgccaca gtgtgttctt tggtcgaatt tgacgaagaa aacaagccac 180  
 ggccaggcac aattattcca 200

<210> 116  
 <211> 471  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 116  
 tttggtcgaa aaaagactgc tactgctgtg gcatattcca aaaagggaaa aggattaatc 60  
 aagggcaatg gccgtccttt agaatttttg caacctgaaa ttcttcgtat taagctacaa 120  
 gagccattgt tgattgtagg aaaggacaaa tttgctggaa tggatattcg catccgtgtc 180  
 aaagggtggtg gtcattgtgc acaaatttat gcaattcgac agtcaattgc taaagttttg 240  
 gtggcctatt accagaaaaa cgtggatgag caaagcaaga aagaattgaa ggatcaactt 300  
 gttgcttatg atcgttaattt gcttgttggc gatccgagac gtcacgagcc aaagaagttt 360  
 ggaggacctg gtgctcgtgc tcgttatcag aaatcttatc gttagaagat atgaaattat 420  
 aaaattgtgt gttacgaatt aattgttatt ttgttgggat aaatntgaat a 471

<210> 117  
 <211> 593  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 117  
 gaattcaaaa aatattaaaa ttgtttaata taatttctaa aatgaagcca aaggttggaa 60

## AKK110P1

ttaacggatt	tggacgtatt	ggacgtcttg	ccctgcgtgc	agcggtcgag	aaggatactg	120
tccaagttgt	ggctgtcaat	gacccgttca	ttgatcttga	ctatatgggc	tatatgttta	180
actatgattc	caccacgga	cgctttaaag	gaaagattca	agcaagcaat	ggaaatttgg	240
tagttgagaa	ggagggaaag	tctactcata	ctatcaaagt	tttcaacttc	aaagaacctg	300
aaaagattga	ctgggcaggt	tctggtgctg	attttgttat	tgagtcgact	ggagttttta	360
ctactaccga	gaaagcttct	gctcacttga	agggcggagc	caagaaagtg	gttatctccg	420
ctccatctgc	tgatgctcca	atgtttgtgg	ttggtgttaa	tgaggacaaa	tatgatcctt	480
ccaagcatca	tatcattagt	aatgcttctt	gcactactaa	ttgtcttgct	cctcttgcca	540
aggttataaa	tgacgagttt	ggcataattg	aaagtgaat	gactactgga	cac	593

&lt;210&gt; 118

&lt;211&gt; 576

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 118

gaattccgag	tttttttttt	ttttttttta	aacaaaaaatt	aaaagattta	tcgccatcct	60
ttgccagcca	tttggccgcc	atttttttgt	gcacaataaa	tttttttgta	atttttgggg	120
tgagggggaa	gtaaaatgaa	agaagggaga	gagatatgaa	ttggagggtt	ttttgttaa	180
ataaattttt	ttttcttgaa	aattcttccc	gtttctgagc	ttttcgtct	tttttcaatt	240
ttcgtttgtc	gaaatactaa	actttacaat	ttggttaggt	tctattttgt	aaacataaat	300
atctccatta	tcgctgattg	caagggcatg	ggcgttttcg	agaccctttg	caaagctatt	360
agcccttctt	gtgttcatat	ccattacgaa	aacttgggat	tctaattgac	tgccctgatc	420
ttgattgggt	acgccgacga	ggaagtgttc	tttctctcgg	atagcaaaga	ctcgcccaat	480
attttcagcc	tttgtgaaga	aagtgcctgt	ggggacgtaa	gcacgtctat	gttgggtgtg	540
agcgccttct	aatccagcag	aaaagcattg	aatacg			576

&lt;210&gt; 119

&lt;211&gt; 559

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 119

acgcagagta	agttgagatc	ttcaataagg	gttagagagt	gtggtacgag	gaattctcca	60
tttttggtgt	tttcaactgga	gtcaggcttc	ccaaattgac	tgagcaattt	cccattccttg	120
tcaaaactca	ctattcggct	attacagtaa	ccatctgcca	cgaaaaactc	tcctgtactg	180
gcaatagcaa	cgtctgtagg	tttgcaaaaa	tgtttgcatt	ctgtccctgg	aacaagcttt	240
tcgcccaaac	tcataattaa	tttaaaatcc	ttgtcaagtt	tggtgacttg	atgacttcca	300
acgtcagtaa	cccaactatt	gccgtgggca	tcgattgtta	gtccatgagg	catgtaaaac	360
atgctttttc	cgtattcttc	caagactgcc	cctgattccg	tgctataaac	agcaattggt	420
gtgtttgaaa	tgatgccccag	ggatctgttt	aggtggttgt	tctcatcaaa	cgaaaattca	480
tcccaaaactc	tgtcagatcg	gtgaaaaaga	acaagtgcgt	tcaatggatc	caatgcaata	540
cccggagcctt	gccaatat					559

&lt;210&gt; 120

&lt;211&gt; 366

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 120

tttaagaatt	ttttaaaaaat	taaaacttgg	actagatttt	aataaaatgt	cagctccacg	60
tagtgttgct	agcgggtgtg	gtgctgctgt	tatgaataag	caagcaagta	aatacaatga	120
agttgaaggga	gaactccttc	ttaattggat	taagaaagtg	acaggcgaaa	atattgctat	180
aaacggaact	agggaaaatt	ttgtgaaaca	attgaaagat	ggaactctgc	tctgcaaat	240
tgctaacaaa	attgtgccaa	attcaatcac	aaaggcacag	gcaaaaccga	acagcacatt	300
ccaatatatg	agcaatttgg	agctgttctt	aacattttatt	tcaagccaag	gagtccttag	360
ggagga						366

&lt;210&gt; 121

&lt;211&gt; 661

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 121

## AKK110P1

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ttagttgaat ctcgtgacct ctactctggt tgtatgacat taaattctct tggccgcatt 60
ttggaacgct aaggaaaaac tcattccagag cagggttaagt cgtcagaaat tcttaatttg 120
ggtactggag accaagtgcg cttctgtggt taaagatggg aaattgaaag aatttttggt 180
aaacataata aaaagacatt ttatggcaat aaaaaaatgt caaaaaagct tgtcttttaa 240
atattttggc aaaacatttt actttcacaa aatttttaaaa taaatttatg aagattgttc 300
cgtcactttc atcattttccg atcgaccttt gttgttttct aagttcgttg gccaaagaaa 360
ggatatgtaa aattgaatta tgaataaaaa taaatcactc aatcagaggc attgttagtc 420
tctcactttc tctcttttac ccattggcta accagcttta aggatttttt ccataagttc 480
aagggtgtacg taaatcgaat accgactgtg gtatcttaat ttttccatga aattctccaa 540
taaaaaaaaaa ttttttttat tttttttcca taatgctatc tatatttttt gcttttaatc 600
ttttttggct atcaggcctt aaaatagtaa atatacttat attaataattt tatttccttt 661
a

```

<210> 122  
 <211> 173  
 <212> DNA  
 <213> Meloidogyne incognita

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<400> 122
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gggaaattgc tcagtcaatt tgggaagcct gactccagtg aaacacccaa aaatggagaa 120
ttccttgtag cacactctct aaccctcatt gaagatctca acttactttg tgt 173

```

<210> 123  
 <211> 584  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 123
cgcattcaat gctttttctgc tggattagaa ggcgctcaac accaacaatag acgtgcttac 60
gtcccccacag gcacttttctt cacaaggctt gaaaatattg ggcgagtcctt tgctatccga 120
gagaaagaac acttctctct cggcgctcacc aatcaagatc agggcagtcga attagaatcc 180
caagttttcg taatggatat gaacacagga agggctaata gctttgctaa ggggtctagaa 240
aacgcccattg cccttgcaat cagcgataat ggagatatatt atgtttcaca aatagaaccc 300
aaccaaaattg taaaatttag tatctcgaca aacgaaaatt gagaaaaaaa aaaaaaaagc 360
tcagaaaacgg gaagaatttt caagaaaaaaa tttttttacc aaacaaaaaaa cctccaattc 420
atactctccc ctcttttcat ttttcttccc ccttctcccc aaaaattaca aaaaatttta 480
ttgtgcacaa aaaaatgggc gggcgggcga atggctgggc aaaggatggc gataaatctt 540
ttaatttttg aaaaaaaaaa aaagaattcg aattatatgg ccta 584

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<210> 124  
 <211> 650  
 <212> DNA  
 <213> Meloidogyne incognita

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<400> 124
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atgttatcga gaagatcgag gctgggtaca aaaagttgca ggaggcaccg gagtgcaggt 120
ctcttctcaa gaagtacttc acgaaggaaag ttatggacca gtgtaaaggg ctcaaaacta 180
agcttggtag gaacttgctt gatgtgatcc actctggagt tgcgaatctc gatagcgggt 240
ttgtgtttta tgcgcctgat gctgagtcct acactctctt caaaccgctt tttgaccgga 300
ttattcagga ttaccacaat ggatttgac ctgaccagaa gcagccgcaa actgacttgg 360
gtgaggggaaa gactcagctt ttgcctgatc tggatcctga gggtaaattc atcaactcga 420
ctcgtgttcg atgtgggcgt tctcttcagg gatatccgtt caatccgtgc ttgactaaag 480
agaattatcag ggaaatgcat gacaaagtta aaggggtttt tgagcagctt aagtctgatg 540
ctgagcttgg tggcacctat tatcctttgg aggggaatgac caaagaggtt caaactcaat 600
tgatcaagga tcacttctct ttcaaagaag gagaccgctt tttgcaagct 650

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<210> 125  
 <211> 1013  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 125

## AKK110P1

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taaatcttct tctttatctt ttaaaaaatt atttcttaaa tttattcttc tcctcttcgt 120
gttttgaatc aaataattaa attttaaaatt atttaaacag ctacacgagg cctcagcctc 180
ccccgttgca ttcaaattgg tcggcacggg tgggcatgat aattttattt tttaggtaat 240
tttggtgaga aaatatcttt aaaggtaata atgtcctttt ggacaattaa aaaaaaactc 300
gaggagagag tgaatatctt tacaaattat ttgaagagca gccagcctat tgttatcaac 360
aaaaaacctt caaaatgccg gaaaatgatt atgatgagga ggaggcgcca aacgccacga 420
tggaacaaca ggtagcttca ggtggacagc caaaacgctg ttggaaaatg gacattatcc 480
cagctgcgcc agactgatgg tataattcca tcccaggccg gttggaacaa gggagactcc 540
caaaagttga tgaccaattt tggtaactca cgtaacacaa caaccaaaat tcgtgctgaa 600
tgccttgctg atgtgcctga agaaattgct cttaaaagtc acggtgaagt acgcctccaa 660
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ttatgcagag aaggagtgtt tgtgagtcaa gaccagccg atttatagcc cctccagaa 780
gagataatcc gtgctagcga tggaaattgtt cgtctcaat ccggtacca caaattcgac 840
tcccaaaagg gaattggtcag ctccggtaca aaccgacgag aaactacaag aatgaaagac 900
accaaacatc cggaaataca ccacgaagtt aacattgacc aaagcgaaat tccttgcaa 960
tctggtacaa acaaattcgc atcccaaaag ggaatgacca gcttcggtac aaa 1013

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&lt;210&gt; 126

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 126

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tggtggacac tgctcaccca gaatacagtc acgaaagcag catcgatcaa acgagcattc 60
cttaccaaat gggatcaaat 80

```

&lt;210&gt; 127

&lt;211&gt; 585

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 127

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agggaaatgac ttgcttttga cagccacggt gggaggtgct tgacccgagc attagctacc 60
agaaccgtaa atcacaagga atggtccgct tccaatccgg aacaaaccgg gtcgcctcgc 120
aagcgggcat gacaggtttt ggaactccaa ggaacacaa acacgaggcg gactctggcg 180
aacttcata cgaagatatg aagaagttag aaacgataat tccatcccag gccggttggg 240
ataagggaga ctctcaaaag ttgatgactg gatttggtag tcctcgtgac gttaaaggca 300
aacatttgaa cgtatttggg gagttggaat acccagagga ggctgaaatt tcgttggatc 360
gactttaaag gaattttaga agagaagaaa gaaaagagaa atttagtggg aggaaggcaa 420
cgacatttga ctctacaatt gacacacacc ttttcacaca tttaaaaaat acattaaaaa 480
aaaatttttt ttggcttttt ggcttgcctc tattttttcc ccccatcatt ctccctattc 540
tctcatttgg atgcaaaactg gaattttaaa aaaaaaaaaa aaaaa 585

```

&lt;210&gt; 128

&lt;211&gt; 287

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 128

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catctggaga aacgttgagg caatacatcg ttattggccg taaacttcct acagagaatg 60
agccaaatcc aaaactttac aaaatgcaaa tttttgccag taatcatgtt gttgctaaat 120
cgcgtttctg gtactttact agtatgttgc gtcgtgttaa gaagactaac ggagagattg 180
tttcgtgtca ggagggtttt gaaaagaaga taggctctgt aaagaattat ggaatttggc 240
ttcgttatga ctctcgaacc ggtcatcaca acatgtaccg tgaatac 287

```

&lt;210&gt; 129

&lt;211&gt; 175

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 129

```

gctgtcactc aggcattatc cgacatgggt gctcgtcatc gtgctcaagc cgatcgaatc 60
caaataatca aggttcaacc gatcaaggct gccgatttga aacgtactgg agttaaacag 120

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## AKK110P1

ttccacaact cttcaatcaa gtttcctttg ccgcatcgtg tgaatgacaa acgtc 175

<210> 130

<211> 599

<212> DNA

<213> Meloidogyne incognita

<400> 130

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aaaggttcac	cttctaagta	aggattgtag	cggctgtatg	attgatgttg	cttttgttgg	120
ggagcaatag	aacgcttgcg	tcgccgaggc	tcctcagccc	tagtaacgtg	aaatttcttt	180
gcaatcatcg	atttgtgtag	tccatttttg	gctaagacct	gttctaagtc	ttgttcatat	240
tgttcagaat	tgctttttga	ttgacagtta	aacatgtgtt	cttggtcaca	aaggcattgc	300
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ttgtcactct	taatctcttg	gcggtgtgaa	aattcagatt	ctggatggag	ttgttgggtc	480
ccttcaccgg	cacctcctgt	cataaattta	tgtccaaacg	caatgggccc	ggaagcattt	540
tcaatgtcac	gagaaatcaa	gtcgattaat	tgtgaatgcg	gaaatatagg	ctccccaga	599

<210> 131

<211> 466

<212> DNA

<213> Meloidogyne incognita

<400> 131

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attggaggat	tgcaagaggc	ttgggagtaa	tacagcacat	gataatgcac	aagttgctcg	180
tgaaaaatgat	gtggtgatta	tagcaggtaa	accaactatt	gtgtctaaag	ttgcttcgga	240
aattgcacca	gccatccgcc	gagatcatgt	acttatttct	atagcattgg	gcatacccat	300
acgctacatt	gagcagtaat	tgacttcaga	atcccgaatt	gttcgtgtaa	tgccagatac	360
tcctgtaggt	ggtaggagca	ggctgctgca	gccatatatc	attgggatca	gcattgtcag	420
gatagggtgat	gcccagatag	ttcaagatct	tctgataacg	ctggggg		466

<210> 132

<211> 266

<212> DNA

<213> Meloidogyne incognita

<400> 132

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tggaattgga	agcgccgttg	aagatttgtg	gcgatattca	cggtaataac	aacgaccttt	120
tgcggtcttt	tgaatatgga	ggttttccgc	ctgaagcgaa	ttattttatt	ttgggtgatt	180
atgtggatag	aggaagcgag	agcttgagaa	cgatttgttt	gctgttgccc	tacaagatca	240
aatcccccca	aaattctttt	tgctga				266

<210> 133

<211> 308

<212> DNA

<213> Meloidogyne incognita

<400> 133

tctatcaacc	gaatatatgg	attttacgat	gaatgcaaac	gcagattttc	tataaaattg	60
tgaaaaacat	ttactgattg	cttcaattgt	ctgccaaattg	ctgctgtgat	cgatgagaaa	120
atattttggt	gccatggagg	tttgtcacca	gattttgcaga	atatggagca	aattcgaaga	180
attatgacgac	cgacggatgt	gccagatata	ggctcttctct	gcgaccttct	atgggtctgat	240
ccagaccaag	atgtccaagg	attgggagaa	aatgatcgtg	gggtctcttt	cacttttggg	300
ccagatgt						308

<210> 134

<211> 335

<212> DNA

<213> Meloidogyne incognita

## AKK110P1

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 tggccgcccc tgatggaaga agcaggcaaa attatttaca agaacattca attcctcaac 120  
 tttttgaggg ttaaatgact ggacttatat acaatcaacc aatcgatcct attcaatttt 180  
 tggagaatgc aatagctaaa cttcgaaaaa atcctgatct tccattaaag tgggatactt 240  
 ttataagtgt ttgcctcaa caacagcaac aacaacagac gagaatgaat actggagaaa 300  
 atgcagtctt ttataaacia agcactccta tcgaa 335

<210> 135  
 <211> 506  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 135  
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 atccacaaac ataattattat tgaacttttc ctttttaaaa ctatcaaaag gccttctttg 120  
 ttcctgagac tttgatcacc ttcaaaacat taaaacgaac agttttactc aaaggcctgc 180  
 attcaccgat cgtgacaata tcaccaatag agatatcacg gaaacatggc gaacagtga 240  
 cggacatgtt tttgtgacgt ttctcgatc gacgatattt cggaaacaaag tgcaataat 300  
 cagccgaat gacaattgtg cgctgcattt tgttcttgat aacaacacca gtcaaaatac 360  
 ggccacgaat tgaacattt ccagtgaag gacactttt gtcaatataa ttgccttcga 420  
 tggcctcgcg tggagtttta aatcctaacc caacattctt ccaataacga tccttatttt 480  
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<210> 136  
 <211> 230  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 136  
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 <213> Meloidogyne incognita

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<210> 139  
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## AKK110P1

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 139

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AKK110P1

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&lt;211&gt; 37

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

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&lt;210&gt; 13

&lt;211&gt; 161

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

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&lt;210&gt; 14

&lt;211&gt; 306

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

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&lt;211&gt; 261

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

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&lt;210&gt; 16

&lt;211&gt; 151

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 16

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&lt;210&gt; 17

&lt;211&gt; 306

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